



NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP DEVELOPMENTAL AND REPRODUCTIVE TOXICITY TECHNICAL REPORT ON THE MODIFIED ONE-GENERATION STUDY OF

BISPHENOL AF (CASRN 1478-61-1)
ADMINISTERED IN FEED TO SPRAGUE
DAWLEY (HSD:SPRAGUE DAWLEY[®] SD[®])
RATS WITH PRENATAL, REPRODUCTIVE
PERFORMANCE, AND SUBCHRONIC
ASSESSMENTS IN F₁ OFFSPRING

NTP DART 08

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**NTP Developmental and Reproductive Toxicity
Technical Report on the
Modified One-Generation Study of
Bisphenol AF (CASRN 1478-61-1)
Administered in Feed to Sprague Dawley
(Hsd:Sprague Dawley[®] SD[®]) Rats with Prenatal,
Reproductive Performance, and Subchronic
Assessments in F₁ Offspring**

DART Report 08

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Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The NTP Technical Report series for developmental and reproductive toxicity (DART) studies began in 2019. The studies described in this NTP Technical Report series (i.e., the NTP DART Report series) are designed and conducted to characterize and evaluate the developmental or reproductive toxicity of selected substances in laboratory animals. Substances (e.g., chemicals, physical agents, and mixtures) selected for NTP reproductive and developmental studies are chosen primarily on the basis of human exposure, level of commercial production, and chemical structure. The interpretive conclusions presented in NTP DART reports are based only on the results of these NTP studies, and extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection for study per se is not an indicator of a substance's developmental or reproductive toxicity potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and the Food and Drug Administration [Good Laboratory Practice Regulations](#) and meets or exceeds all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the [Public Health Service Policy on Humane Care and Use of Laboratory Animals](#). Studies are subjected to retrospective quality assurance audits before they are presented for public review. Draft reports undergo external peer review before they are finalized and published.

NTP DART reports are available free of charge on the [NTP website](#) and cataloged in [PubMed](#), a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's [Chemical Effects in Biological Systems](#) database.

For questions about the reports and studies, please email [NTP](#) or call 984-287-3211.

Table of Contents

Foreword.....	ii
Tables.....	v
Figures.....	vii
About This Report.....	x
Explanation of Levels of Evidence for Reproductive Toxicity	xiv
Explanation of Levels of Evidence for Developmental Toxicity	xvii
Peer Review	xx
Publication Details	xxi
Acknowledgments.....	xxi
Abstract.....	xxii
Modified One-Generation Study.....	xxii
Genetic Toxicology.....	xxiv
Conclusions.....	xxv
Introduction.....	1
Chemical and Physical Properties.....	1
Production, Use, and Human Exposure	1
Regulatory Status	2
Absorption, Distribution, Metabolism, Excretion, and Toxicokinetics	2
Experimental Animals	2
Humans	4
Developmental and Reproductive Toxicity	5
Models of Endocrine Activity.....	5
Experimental Animals	5
Humans	6
General Toxicity.....	6
Experimental Animals	6
Humans	6
Genetic Toxicity.....	6
Study Rationale	7
Materials and Methods.....	9
Overview of Pre- and Postnatal Dose Range-finding and Modified One-Generation Study Designs	9
Procurement and Characterization.....	12
Preparation and Analysis of Dose Formulations.....	12
Animal Source.....	13
Animal Health Surveillance	13
Animal Welfare.....	13
Experimental Design.....	14
Dose Range-finding Study	14

Bisphenol AF, NTP DART 08

Modified One-Generation Study with Prenatal, Reproductive Performance, and Subchronic Cohorts.....	14
Statistical Methods.....	25
Analysis of Fetal Malformations and Variations.....	25
Analysis of Incidences of Gross Pathology and Morphology Findings.....	25
Analysis of Continuous Endpoints.....	26
Analysis of Feed Consumption Data.....	27
Analysis of Gestational and Fertility Indices.....	27
Body Weight Adjustments.....	27
Analysis of Time-to-Event Data.....	27
Analysis of Vaginal Cytology Data.....	28
Historical Control Data.....	28
Quality Assurance Methods.....	29
Genetic Toxicology.....	29
Bacterial Mutagenicity.....	29
Peripheral Blood Micronucleus Test.....	29
Results.....	31
Data Availability.....	31
Dose Range-finding Study.....	31
Maternal Findings.....	31
F ₁ Offspring Findings.....	37
Exposure Concentration Selection Rationale for the Modified One-Generation Study of Bisphenol AF.....	41
Modified One-Generation Study.....	42
F ₀ Generation: Maternal Findings.....	42
F ₁ Generation: Preweaning.....	48
F ₁ Generation: Postweaning through Sexual Maturity.....	53
Developmental Endpoints.....	57
F ₁ Cohort Data.....	67
Prenatal and Reproductive Performance Cohorts: Mating and Fertility.....	67
Prenatal Cohort Findings.....	76
Reproductive Performance Cohort Findings.....	81
F ₁ Necropsies: Prenatal, Reproductive Performance, and Subchronic Cohorts.....	90
Clinical Pathology.....	101
Histopathology.....	102
F ₂ Necropsies.....	119
Genetic Toxicology.....	123
Discussion.....	124
Conclusions.....	130
References.....	131
Appendix A. Chemical Characterization and Dose Formulation Studies.....	A-1
Appendix B. Ingredients, Nutrient Composition, and Contaminant Levels in 5K96 Rat Ration.....	B-1

Appendix C. Sentinel Animal ProgramC-1
 Appendix D. Genetic Toxicology D-1
 Appendix E. Supplemental Data.....E-1

Tables

Summary of Exposure-related Findings in Rats in the Modified One-Generation Study of Bisphenol AF xxvi
 Table 1. Key Modified One-Generation Study Design Endpoints11
 Table 2. Experimental Design and Materials and Methods in the Dose Range-finding and Modified One-Generation Studies of Bisphenol AF (Prewaning).....19
 Table 3. Experimental Design and Materials and Methods in the Modified One-Generation Study of Bisphenol AF (Postweaning)24
 Table 4. Summary of Mean Body Weights and Body Weight Gains of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation and Lactation (Dose Range-finding Study)32
 Table 5. Summary of Feed and Test Article Consumption of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation and Lactation (Dose Range-finding Study).....35
 Table 6. Summary of the Reproductive Performance of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation (Dose Range-finding Study)36
 Table 7. Summary of F₁ Litter Size and Pup Survival Following Perinatal Exposure to Bisphenol AF (Dose Range-finding Study).....37
 Table 8. Summary of F₁ Male and Female Pup Mean Body Weights and Body Weight Gains Following Perinatal Exposure to Bisphenol AF (Dose Range-finding Study).....38
 Table 9. Summary of Mean Body Weights and Body Weight Gains of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation43
 Table 10. Summary of Feed and Test Article Consumption of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation44
 Table 11. Summary of the Reproductive Performance of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation45
 Table 12. Summary of Mean Body Weights, Body Weight Gains, and Feed and Test Article Consumption of F₀ Female Rats Exposed to Bisphenol AF in Feed during Lactation46
 Table 13. Summary of F₁ Litter Size and Pup Survival Following Perinatal Exposure to Bisphenol AF49
 Table 14. Summary of F₁ Male and Female Pup Mean Body Weights and Body Weight Gains Following Perinatal Exposure to Bisphenol AF51
 Table 15. Summary of Postweaning Mean Body Weights, Body Weight Gains, and Feed and Test Article Consumption of All F₁ Male Rats Exposed to Bisphenol AF in Feed.....54
 Table 16. Summary of Postweaning Mean Body Weights, Body Weight Gains, and Feed and Test Article Consumption of All F₁ Female Rats Exposed to Bisphenol AF in Feed.....56

Bisphenol AF, NTP DART 08

Table 17. Summary of Anogenital Distance of F ₁ and F ₂ Male and Female Rats Exposed to Bisphenol AF in Feed	57
Table 18. Summary of Testicular Descent of F ₁ and F ₂ Male Rats Exposed to Bisphenol AF in Feed	59
Table 19. Summary of Vaginal Opening of F ₁ and F ₂ Female Rats Exposed to Bisphenol AF in Feed	61
Table 20. Summary of Balanopreputial Separation of F ₁ and F ₂ Male Rats Exposed to Bisphenol AF in Feed	64
Table 21. Summary of Estrous Cycle Data and Markov Model Estimates of Estrous Stage Length and 95% Confidence Intervals for All F ₁ and F ₂ Female Rats Exposed to Bisphenol AF in Feed	69
Table 22. Summary of Mating and Fertility Performance of F ₁ Male and Female Rats Exposed to Bisphenol AF in Feed	71
Table 23. Summary of Reproductive System Parameters of F ₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed	71
Table 24. Summary of Gestation Mean Body Weights and Body Weight Gains for F ₁ Female Rats Exposed to Bisphenol AF in Feed	73
Table 25. Summary of Gestation Feed and Test Article Consumption for F ₁ Female Rats Exposed to Bisphenol AF in Feed	75
Table 26. Summary of Uterine Content Data for F ₁ Females in the Prenatal Cohort Exposed to Bisphenol AF in Feed	77
Table 27. Summary of Head Findings in Fetuses Exposed to Bisphenol AF in Feed	79
Table 28. Summary of Select Skeletal Findings in Fetuses Exposed to Bisphenol AF in Feed	80
Table 29. Summary of Reproductive Parameters of F ₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed	82
Table 30. Summary of Mean Body Weights, Body Weight Gains, and Feed and Test Article Consumption of F ₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed during Lactation	82
Table 31. Summary of F ₂ Litter Size and Pup Survival Following Perinatal Exposure to Bisphenol AF	84
Table 32. Summary of F ₂ Male and Female Pup Mean Body Weights and Body Weight Gains Following Perinatal Exposure to Bisphenol AF	86
Table 33. Summary of Postweaning Mean Body Weights, Body Weight Gains, and Feed and Test Article Consumption of All F ₂ Male and Female Rats Exposed to Bisphenol AF in Feed	87
Table 34. Summary of Gross Necropsy Findings in Adult F ₁ Male Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed	92
Table 35. Summary of Gross Necropsy Findings in Adult F ₁ Male Rats in the Prenatal and Reproductive Performance Cohorts Exposed to Bisphenol AF in Feed	92
Table 36. Summary of Organ Weights of Adult F ₁ Male Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed	93
Table 37. Summary of Organ Weights of Adult F ₁ Male Rats in the Prenatal and Reproductive Performance Cohorts Exposed to Bisphenol AF in Feed	95
Table 38. Summary of Gross Necropsy Findings in Adult F ₁ Female Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed	98

Table 39. Summary of Gross Necropsy Findings in Adult F ₁ Female Rats in the Prenatal and Reproductive Performance Cohorts Exposed to Bisphenol AF in Feed.....	98
Table 40. Summary of Organ Weights of Adult F ₁ Female Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed	99
Table 41. Summary of Ovary Weights of Adult F ₁ Female Rats in the Prenatal and Reproductive Performance Cohorts Exposed to Bisphenol AF in Feed.....	100
Table 42. Summary of Select Hematology Data for F ₁ Adult Female Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed.....	101
Table 43. Summary of Select Clinical Chemistry Data for F ₁ Male and Female Adult Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed.....	102
Table 44. Incidences of Select Nonneoplastic Lesions in Adult F ₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed	103
Table 45. Incidences of Select Nonneoplastic Lesions in Adult F ₁ Male Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed.....	104
Table 46. Incidences of Select Nonneoplastic Lesions in Adult F ₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed	112
Table 47. Incidences of Select Nonneoplastic Lesions in Adult F ₁ Female Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed.....	113
Table 48. Summary of Reproductive System Parameters of F ₂ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed	119
Table 49. Summary of Gross Necropsy Findings in F ₂ Male Rats Exposed to Bisphenol AF in Feed	120
Table 50. Summary of Organ Weights from F ₂ Male Rats Following Perinatal Exposure to Bisphenol AF.....	121
Table 51. Summary of Organ Weights from F ₂ Female Rats Following Perinatal Exposure to Bisphenol AF.....	123

Figures

Figure 1. Bisphenol AF (CASRN 1478-61-1; Chemical Formula: C ₁₅ H ₁₀ F ₆ O ₂ ; Molecular Weight: 336.23).....	1
Figure 2. Metabolism of Bisphenol AF in Rodents	4
Figure 3. Design of a Dose Range-finding Study	9
Figure 4. Design of a Modified One-Generation Rat Study	10
Figure 5. Growth Curves for F ₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation and Lactation (Dose Range-finding Study)	34
Figure 6. Lactation Growth Curves for F ₁ Male Pups Following Perinatal Exposure to Bisphenol AF (Dose Range-finding Study)	40
Figure 7. Lactation Growth Curves for F ₁ Female Pups Following Perinatal Exposure to Bisphenol AF (Dose Range-finding Study)	40
Figure 8. Design of the Modified One-Generation Study – F ₀ Generation	42
Figure 9. Growth Curves for F ₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation.....	44
Figure 10. Growth Curves for F ₀ Female Rats Exposed to Bisphenol AF in Feed during Lactation.....	47
Figure 11. Design of the Modified One-Generation Study – F ₁ Generation: Prewaning	48

Figure 12. Lactation Growth Curves for F ₁ Male Pups Following Perinatal Exposure to Bisphenol AF	52
Figure 13. Lactation Growth Curves for F ₁ Female Pups Following Perinatal Exposure to Bisphenol AF	52
Figure 14. Design of the Modified One-Generation Study – F ₁ Generation: Postweaning.....	53
Figure 15. Postweaning Growth Curves for All F ₁ Male Rats Exposed to Bisphenol AF in Feed.....	55
Figure 16. Postweaning Growth Curves for All F ₁ Female Rats Exposed to Bisphenol AF in Feed.....	57
Figure 17. Time to Testicular Descent of F ₁ and F ₂ Male Offspring Exposed to Bisphenol AF in Feed.....	60
Figure 18. Time to Vaginal Opening of F ₁ Female Offspring Exposed to Bisphenol AF in Feed.....	62
Figure 19. Time to Vaginal Opening of F ₂ Female Offspring Exposed to Bisphenol AF in Feed.....	63
Figure 20. Time to Balanopreputial Separation of F ₁ Male Offspring Exposed to Bisphenol AF in Feed.....	65
Figure 21. Time to Balanopreputial Separation of F ₂ Male Offspring Exposed to Bisphenol AF in Feed.....	66
Figure 22. Design of the Modified One-Generation Study – Prenatal and Reproductive Performance Cohorts.....	67
Figure 23. Markov Model Estimates of Stage Lengths and 95% Confidence Intervals for F ₁ Female Rats Exposed to Bisphenol AF in Feed	70
Figure 24. Markov Model Estimates of Stage Lengths and 95% Confidence Intervals for F ₂ Female Rats Exposed to Bisphenol AF in Feed	70
Figure 25. Gestation Growth Curves for F ₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed.....	74
Figure 26. Gestation Growth Curves for F ₁ Female Rats in the Prenatal Cohort Exposed to Bisphenol AF in Feed	74
Figure 27. Design of the Modified One-Generation Study – Prenatal Cohort	76
Figure 28. Design of the Modified One-Generation Study – Reproductive Performance Cohort.....	81
Figure 29. Lactation Growth Curves for F ₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed.....	83
Figure 30. Lactation Growth Curves for F ₂ Male Pups Following Perinatal Exposure to Bisphenol AF	88
Figure 31. Postweaning Growth Curves for All F ₂ Male Rats Exposed to Bisphenol AF in Feed.....	88
Figure 32. Lactation Growth Curves for F ₂ Female Pups Following Perinatal Exposure to Bisphenol AF	89
Figure 33. Postweaning Growth Curves for All F ₂ Female Rats Exposed to Bisphenol AF in Feed.....	89
Figure 34. Representative Images of Germinal Epithelial Degeneration in the Testis of F ₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)	105

Bisphenol AF, NTP DART 08

Figure 35. Representative Images of Leydig Cell Atrophy in the Testis of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E).....106

Figure 36. Representative Images of Duct Atrophy and Hypospermia in the Epididymis of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)108

Figure 37. Representative Image of Exfoliated Germ Cells in the Ducts of the Epididymis of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)109

Figure 38. Representative Images of Hypoplasia in the Prostate Gland, Seminal Vesicle, Coagulating Gland, and Cowper’s Gland of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)110

Figure 39. Representative Images of Hypoplasia in the Levator Ani/bulbocavernosus (LABC) Muscle Complex of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)111

Figure 40. Representative Images of Hypoplasia in the Ovary of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)114

Figure 41. Representative Images of Hypoplasia in the Uterus of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)116

Figure 42. Representative Image of Epithelial Squamous Metaplasia in the Uterus of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)117

Figure 43. Representative Image of Cystic Glandular Dilation in the Uterus of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E).....117

Figure 44. Representative Images of Endometrial Stromal Hyalinization in the Uterus of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)118

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Explanation of Levels of Evidence for Reproductive Toxicity

The National Toxicology Program (NTP) describes the results of individual studies of chemical agents and other test articles and notes the strength of the evidence for conclusions regarding each study. Generally, each study is confined to a single laboratory animal species, although in some instances, multiple species may be investigated under the purview of a single study report. Negative results, in which the study animals do not exhibit evidence of reproductive toxicity, do not necessarily imply that the test article is not a reproductive toxicant, but only that exposure to the test article did not result in reproductive toxicity under the specific conditions of this study. Positive results demonstrating that the test article causes reproductive toxicity in laboratory animals under the study conditions are assumed relevant to humans, unless data are available that demonstrate otherwise. In addition, such positive effects are assumed to be primary effects, unless there is clear evidence that they are secondary consequences of excessive toxicity to nonreproductive organ systems. Given that developmental events are interrelated in the reproductive process, developmental toxicity may be detected in reproductive studies. Evaluation of such adverse effects on development should be based on the criteria outlined in the Levels of Evidence for Evaluating Developmental Toxicity.

It is critical to recognize that the “level-of-evidence” categories herein only identify whether exposure to the test article is a reproductive **hazard**. The determination of any **risk** to humans from the test article requires data on human exposure and is not part of hazard identification.

Five categories are used to differentiate the strength of the evidence for reproductive toxicity observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major design or performance flaws (**inadequate study**). Application of these criteria requires professional judgment by individuals with ample experience and understanding of the animal models and study designs employed. For each study, the findings are evaluated to determine the appropriate level-of-evidence category and a conclusion statement is prepared that describes the findings supporting that category. Separate conclusion statements may be prepared for males and females. The level-of-evidence categories refer to the strength of the evidence of the experimental results and not to potency or mechanism.

Levels of Evidence for Evaluating Reproductive Toxicity

- **Clear evidence** of reproductive toxicity is demonstrated by data that indicate an exposure-related effect of the test article on fertility or fecundity, or by changes in multiple interrelated reproductive parameters of sufficient magnitude that by weight of evidence implies a compromise in reproductive function.
- **Some evidence** of reproductive toxicity is demonstrated by data that indicate exposure-related effects of the test article on reproductive parameters, the outcome of which is judged by weight of evidence to have potential to compromise reproductive function. Relative to *clear evidence of reproductive toxicity*, such effects would be characterized by greater uncertainties or weaker relationships with regard to dose of test article and/or the severity, magnitude, incidence, persistence, and/or decreased concordance among affected endpoints.

- **Equivocal evidence** of reproductive toxicity is demonstrated by marginal or discordant effects on reproductive parameters that may or may not be related to exposure to the test article.
- **No evidence** of reproductive toxicity is demonstrated by data from a study with appropriate experimental design and conduct that are interpreted as showing no biologically relevant effects on reproductive parameters related to exposure to the test article.
- **Inadequate study** of reproductive toxicity is demonstrated by a study that, because of major design or performance flaws, cannot be used to determine the occurrence of reproductive toxicity.

Note: The term exposure-related describes any exposure-response relationship, recognizing that the test article-related responses for some endpoints may be non-monotonic due to saturation of exposure or effect, overlapping exposure-response behaviors, changes in immunologic manifestations at different exposure levels, or other phenomena.

When the level-of-evidence category for a particular study is selected, consideration must be given to key factors that would support that selection. Such consideration should allow for incorporating scientific experience and current understanding of reproductive toxicity studies in laboratory animals, particularly with respect to interrelationships between endpoints, impact of changes on reproductive function, relative sensitivity of endpoints, normal background incidence, and specificity of the effect. For evaluations for which it is difficult to choose between adjacent level-of-evidence categories, the following factors should be considered to help inform decision-making:

- Increases in severity and/or prevalence (more individuals and/or more affected litters) as a function of dose of the test article generally strengthens the level of evidence, keeping in mind that the specific manifestation of effect may be different with increasing dose. For example, histological changes at a lower dose may reflect reductions in fertility at higher doses.
- In general, the more animals affected, the stronger the evidence; however, effects in a small number of animals across multiple, related endpoints should not be discounted, even in the absence of statistical significance. In addition, effects with low background incidence, when interpreted in the context of historical controls, may be biologically important.
- Consistency of effects across generations in a multigenerational study may support a higher level of evidence. However, special consideration should be given when decrements in reproductive outcomes are found in the F₁ generation that were not seen in the F₀ generation, as this may suggest both developmental and reproductive toxicity. Alternatively, if effects are observed in the F₁ generation and not in the F₂ generation (or the effects occur at a lower frequency in the F₂ generation), this outcome may be due to survivor selection for resistance to the effect (i.e., if the effect is incompatible with successful reproduction or development, then the affected individuals will not produce offspring).
- Transient changes (e.g., pup weight decrements) by themselves may be weaker indicators of an effect than persistent changes.

Bisphenol AF, NTP DART 08

- Changes in single endpoints by themselves may be weaker indicators of an effect than concordant effects on multiple, interrelated endpoints.
- Marked changes in multiple reproductive tract endpoints without effects on integrated reproductive function (i.e., fertility and fecundity) may be sufficient to reach a conclusion of *clear evidence of reproductive toxicity*.
- Insights from supportive studies (e.g., toxicokinetics, computational models, structure-activity relationships, and studies of absorption, distribution, metabolism, and excretion) and reproductive findings from other in vivo animal studies (conducted by NTP or others) should be drawn upon when interpreting the biological plausibility of an effect.
- New assays or techniques need to be characterized appropriately to build confidence in their utility. Their usefulness as indicators of effect is increased if they are associated with changes in traditional endpoints.

For more information visit: <http://ntp.niehs.nih.gov/go/10003>.

Explanation of Levels of Evidence for Developmental Toxicity

The National Toxicology Program (NTP) describes the results of individual studies of chemical agents and other test articles and notes the strength of the evidence for conclusions regarding each study. Generally, each study is confined to a single laboratory animal species, although in some instances, multiple species may be investigated under the purview of a single study report. Negative results, in which the study animals do not exhibit evidence of developmental toxicity, do not necessarily imply that the test article is not a developmental toxicant, but only that exposure to the test article did not result in developmental toxicity under the specific conditions of this study. Positive results demonstrating that the test article causes developmental toxicity in laboratory animals under the study conditions are assumed relevant to humans, unless data are available that demonstrate otherwise. In addition, such positive effects are assumed to be primary effects, unless there is clear evidence that they are secondary consequences of excessive maternal toxicity. Given that developmental events are interrelated in the reproductive process, reproductive toxicity may be detected in developmental studies. Evaluation of such adverse effects on reproduction should be based on the criteria outlined in the Levels of Evidence for Evaluating Reproductive Toxicity.

It is critical to recognize that the “level-of-evidence” categories herein only identify whether exposure to the test article is a developmental **hazard**. The determination of any **risk** to humans from the test article requires data on human exposure and is not part of hazard identification.

Five categories are used to differentiate the strength of the evidence for developmental toxicity observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major design or performance flaws (**inadequate study**). Application of these criteria requires professional judgment by individuals with ample experience and understanding of the animal models and study designs employed. For each study, the findings are evaluated to determine the appropriate level-of-evidence category and a conclusion statement is prepared that describes the findings supporting that category. Separate conclusion statements may be prepared for males and females. The level-of-evidence categories refer to the strength of the evidence of the experimental results and not to potency or mechanism.

Levels of Evidence for Evaluating Developmental Toxicity

- **Clear evidence** of developmental toxicity is demonstrated by data that indicate an exposure-related effect of the test article that is not secondary to overt maternal toxicity on one or more of the following four elements: embryo-fetal death, structural malformations, growth retardation, or functional deficits.
- **Some evidence** of developmental toxicity is demonstrated by data that indicate exposure-related effects of the test article on one or more of the following four elements: embryo-fetal death, structural malformations, growth retardation, or functional deficits. Relative to *clear evidence of developmental toxicity*, such effects would be characterized by greater uncertainties or weaker relationships with regard to dose of the test article and/or the severity, magnitude, incidence, persistence, and/or decreased concordance among affected endpoints.

- **Equivocal evidence** of developmental toxicity is demonstrated by marginal or discordant effects on developmental parameters that may or may not be related to exposure to the test article.
- **No evidence** of developmental toxicity is demonstrated by data from a study with appropriate experimental design and conduct that are interpreted as showing no biologically relevant effects on developmental parameters related to exposure to the test article.
- **Inadequate study** of developmental toxicity is demonstrated by a study that, because of major design or performance flaws, cannot be used to determine the occurrence of developmental toxicity.

Note: The term exposure-related describes any exposure-response relationship, recognizing that the test article-related responses for some endpoints may be non-monotonic due to saturation of exposure or effect, overlapping exposure-response behaviors, changes in immunologic manifestations at different exposure levels, or other phenomena.

When the level-of-evidence category for a particular study is selected, consideration must be given to key factors that would support that selection. Such consideration should allow for incorporating scientific experience and current understanding of developmental toxicity studies in laboratory animals, particularly with respect to interrelationships between endpoints, impact of changes on development, relative sensitivity of endpoints, normal background incidence, and specificity of the effect. For evaluations for which it is difficult to choose between adjacent level-of-evidence categories, the following factors should be considered to help inform decision-making:

- Increases in severity and/or prevalence (more individuals and/or more affected litters) as a function of dose of the test article generally strengthens the level of evidence, keeping in mind that the specific manifestation of effect may be different with increasing dose. For example, malformations may be observed at a lower dose, but higher doses may produce embryo-fetal death.
- In general, the more animals affected, the stronger the evidence; however, effects in a small number of animals across multiple, related endpoints should not be discounted, even in the absence of statistical significance. In addition, rare malformations with low incidence, when interpreted in the context of historical controls, may be biologically important.
- Consistency of effects across generations in a multigenerational study may support a higher level of evidence. However, if effects are observed in the F₁ generation and not in the F₂ generation (or the effects occur at a lower frequency in the F₂ generation), this outcome may be due to survivor selection for resistance to the effect (i.e., if the effect is incompatible with successful reproduction or development, then the affected individuals will not produce offspring).
- Effects seen in many litters may provide stronger evidence than effects confined to one or a few litters, even if the incidence within those litters is high.
- Because of the complex relationship between maternal physiology and development of the offspring, effects in the embryo-fetus or pup, which are observed at a lower

dose than a dose that induces maternal toxicity, would normally strengthen the evidence for developmental toxicity.

- Concordant effects (syndromic) may strengthen the evidence of developmental toxicity. Changes in single endpoints by themselves may be weaker indicators of an effect than concordant effects on multiple, interrelated endpoints.
- To be designated *clear evidence of developmental toxicity*, the endpoint(s) evaluated should normally show a statistical increase in the deficit, or syndrome, on a litter basis.
- Transient changes (e.g., pup weight decrements, reduced ossification in fetuses) by themselves may be weaker indicators of an effect than persistent changes.
- Uncertainty about the occurrence of developmental toxicity in one study may be lessened by developmental toxicity (even if not identical in effects) observed in a second species.
- Insights from supportive studies (e.g., toxicokinetics, computational models, structure-activity relationships, and studies of absorption, distribution, metabolism, and excretion) and developmental findings from other in vivo animal studies (conducted by NTP or others) should be drawn upon when interpreting the biological plausibility of an effect.
- New assays and techniques need to be characterized appropriately to build confidence in their utility. Their usefulness as indicators of effect is increased if they are associated with changes in traditional endpoints.

For more information visit: <http://ntp.niehs.nih.gov/go/10003>.

Peer Review

The National Toxicology Program (NTP) conducted a peer review of the draft *NTP Developmental and Reproductive Toxicity Technical Report on the Modified One-Generation Study of Bisphenol AF (CASRN 1478-61-1) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats with Prenatal, Reproductive Performance, and Subchronic Assessments in F₁ Offspring* by letter in December 2021 and January 2022 by the experts listed below. Reviewer selection and document review followed established NTP practices. The reviewers were charged to:

- (1) Review and evaluate the scientific and technical elements of the study and its presentation.
- (2) Determine whether the study's experimental design, conduct, and findings support NTP's conclusions under the conditions of this study.

NTP carefully considered reviewer comments in finalizing this report.

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Abstract

Bisphenol AF (BPAF) is used as a curing or crosslinking agent in the processing of fluorocarbon elastomers, rubber processing, and specialty polymers due to its material characteristics, including thermal stability, chemical resistance, and compression set resistance, which are useful in plastics manufacturing and other fabrication processes.

BPAF was selected for evaluation based on a review of compounds that are potentially endocrine-active after concerns were raised about possible effects of bisphenol A (BPA) on the brain, behavior, and prostate gland of fetuses, infants, and children at current human exposure levels. The review assessed a number of agents that could have endocrine activity and are either persistent in the environment or have high human exposures, including chemicals that are structurally related to BPA. BPAF was selected because of its potential for endocrine activity, lack of adequate toxicity data, and potential environmental persistence due to the presence of fluorine atoms.

The objective of the present study was to characterize the potential for BPAF to adversely affect any phase of rat development, maturation, and ability to reproduce. The potential for BPAF to induce subchronic toxicity in the F₁ generation, adversely affect the ability of the F₁ generation to reproduce viable F₂ offspring, and adversely affect F₂ embryo-fetal development was assessed in Sprague Dawley (Hsd:Sprague Dawley[®] SD[®]) rats administered BPAF in 5K96 feed, a diet low in phytoestrogens, using the National Toxicology Program (NTP) modified one-generation (MOG) study design.

NTP conducted a dose range-finding study with exposure concentrations of 0, 937.5, 1,875, 3,750, 7,500, and 15,000 ppm and based on findings of maternal and pup toxicity (significantly decreased body weights) observed at $\geq 7,500$ ppm, exposure concentrations of 338, 1,125, and 3,750 ppm were selected for the MOG study.

Modified One-Generation Study

F₀ dietary exposure began on gestation day (GD) 6 and continued throughout the study. Biological samples were collected on GD 18 (maternal and fetal), on lactation day (LD) 4 (maternal), and on postnatal day (PND) 4 (pup) to determine maternal transfer. At weaning on PND 28, offspring were randomly assigned to the reproductive performance (1/sex/litter), prenatal (1/sex/litter), subchronic (1/sex/litter from 10 litters), or biological sampling (6/sex for sample collection on PND 28 to determine internal concentrations of BPAF and up to 12 females for sample collection at vaginal opening) cohort. Upon sexual maturity, F₁ mating and pregnancy indices were evaluated. In the prenatal cohort, F₂ prenatal development (litter size, fetal weight, and morphology) was assessed on GD 21. In the reproductive performance cohort, littering indices and F₂ viability and growth were assessed until PND 91. The likelihood of identifying potential BPAF-induced adverse effects and their similarity and magnitude—at any phase of growth or development—was increased by examining related endpoints in multiple pups within a litter throughout life, across cohorts, and across generations.

In this study, dietary consumption of BPAF was associated with lower F₀, F₁, and F₂ mean body weights. The lower F₀ female mean body weights and body weight gains during gestation were associated with a significant decrease in PND 1 F₁ pup weights (9% and 15% in the 1,125 and 3,750 ppm groups, respectively) that continued through PND 98. Significant decreases in F₂ mean body weights were also observed for 1,125 ppm male and female pups (12% on PND 28

for both males and females) through weaning, but only female postweaning mean body weights were significantly decreased through PND 91 for both the 338 and 1,125 ppm groups.

Several biochemical and hematological changes in the F₁ generation subchronic cohort were noted. BPAF exposure related changes included significant decreases in serum cholesterol concentrations in both sexes and in serum bile acid concentrations in males, while significant increases in serum triglyceride concentrations were noted in females. Hematological changes were limited to females and included significant decreases in erythrocyte count, hemoglobin concentration, and total white blood cell count.

BPAF-related changes in reproductive performance were observed at all exposure concentrations. For the 3,750 ppm group, a complete absence of pregnant females in the F₁ generation resulted in only two concentration groups for evaluation in the F₂ generation (338 and 1,125 ppm). The majority (89%) of females in the 3,750 ppm group were not cycling and were in persistent estrus. A slight but significant increase in gestation length for F₀ females and a significant decrease in F₁ pup survival (PND 1–4) were also attributed to BPAF exposure. Similar findings, although to a lesser extent, were observed at lower concentrations in the prenatal cohort and included a significant decrease in the number of F₁ females with live fetuses or live litters, number of corpora lutea, and number of implantation sites in the 1,125 ppm group, which were associated with a significant increase in pre- and postimplantation loss values. Significant decreases in the number of corpora lutea and implantation sites were also noted for the prenatal cohort females in the 338 ppm group. Changes in organ weights were also observed in the F₁ generation. In the subchronic cohort, significant increases in the relative weights of the lungs, adrenal glands, and thyroid gland were noted in the 3,750 ppm F₁ males. Significant decreases in relative weights for the liver and kidney (left) were also observed at 3,750 ppm for F₁ males and microscopic findings were observed in the male kidney (mineral lesions along the junction of the cortex and medulla). In F₁ males, lower absolute weights of the dorsolateral prostate, ventral prostate, and seminal vesicles with coagulating glands were observed in the 1,125 and 3,750 ppm groups and of the Cowper's gland and levator ani/bulbocavernosus muscle (LABC) in the 3,750 ppm group. The organ weight changes in the 3,750 ppm group were more than the magnitude of the reductions in body weight and, along with histopathology observations of hypoplasia, indicated a potential direct BPAF-mediated suppression of maturation of these tissues. F₂ males exhibited similar findings in the same reproductive tissues as F₁ males in the 338 and 1,125 ppm groups. Changes in reproductive organ weights that appeared secondary to the effect of BPAF on body weight were limited to lower absolute weights of the testes, epididymides, and preputial glands in all three F₁ exposed groups. The lower testes weights may also be due to direct (germinal epithelium degeneration and Leydig cell atrophy) effects of BPAF exposure. Changes in reproductive organ weights that appear to be secondary to the effect of BPAF on body weight for the F₂ exposed males were limited to the testes and epididymides. Histopathology was not performed on the F₂ generation. BPAF-related changes in andrology parameters were noted in both F₁ and F₂ males.

In F₁ females, reproductive toxicity associated with exposure to BPAF included significant decreases in absolute ovarian and uterus/cervix/vagina weights, with gross observations of reduced size and hypoplasia in the 3,750 ppm group. In the subchronic cohort, significant increases in the relative weights of the thyroid gland and liver were noted in the 3,750 ppm F₁ females. Significant decreases in absolute ovarian weights were also observed in the 338 and 1,125 ppm F₂ females. The magnitude of the reduction in weights of the ovaries in the 1,125 ppm

group was more than the magnitude of the reduction in body weight, suggesting a direct BPAF-mediated suppression of maturation of this tissue.

BPAF-related changes consistent with impaired development include lower mean body weights for all generations, including fetal or pup weights and reduced litter sizes, as well as impacts on fetal parameters and select developmental markers. Developmental landmarks impacted by BPAF exposure included time to vaginal opening (VO), testicular descent, and balanopreputial separation (BPS). No impacts on anogenital distance or areolae and nipple retention were observed in this study. The time to VO was significantly accelerated in all BPAF-exposed groups for both the F₁ and F₂ generations at all exposure concentrations. The mean day of testicular descent was not affected in the F₁ generation, although one male in the 1,125 ppm group and 11 males in the 3,750 ppm group did not attain testicular descent by study termination; however, the mean day of testicular descent was significantly delayed by approximately 2 days for the F₂ offspring in the 1,125 ppm group. In addition, 10 F₁ males in the 3,750 ppm group did not attain BPS. The time to BPS was significantly delayed in both the F₁ and F₂ offspring in the 1,125 and 3,750 ppm groups for the F₁ generation and the 1,125 ppm group for the F₂ generation. BPAF exposure resulted in fetal malformations of the penis and vagina in two F₁ males and three F₁ females in the 3,750 ppm group. Additional findings were limited to an increase in the incidence of dilated and/or misshapen lateral ventricle (brain) in the 1,125 ppm group, which NTP has not recorded in its previous studies, and increases in the incidences of rudimentary and full lumbar I (L1) ribs in the 338 ppm group and rudimentary L1 ribs in the 1,125 ppm group for the prenatal cohort. These last findings were outside the NTP historical control ranges; however, the lack of an exposure-related response impedes a more thorough assessment to determine if they may have been related to BPAF exposure.

As previously reported, average dam daily BPAF intake was estimated on the basis of feed consumption per cage during gestation and lactation for all exposure groups. Average BPAF intake during gestation for F₀ females was lower (26–259 mg BPAF/kg body weight/day [mg/kg/day] when assessed at GD 15–18) than for the lactation period (41–770 mg/kg/day, when measured at LD 1–4). Average BPAF intake estimated around weaning, PND 25–28, was higher than during the earlier part of lactation and was 131, 446, and 1,684 mg/kg/day in the 338, 1,125, and 3,750 ppm groups, respectively. During all three periods, average BPAF intake increased proportionally to exposure concentration. Free (parent only) and total (combined parent and conjugated forms) BPAF concentrations were quantified in maternal plasma and fetuses at GD 18 and maternal and pup plasma at LD 4 and LD 28. Free BPAF F₁ concentrations were higher than corresponding dam concentrations in both GD 18 fetuses and PND 4 pups, demonstrating considerable transfer of BPAF from mother to offspring, whereas total BPAF concentrations were lower than corresponding concentrations in dams, suggesting either preferential transfer of free BPAF and/or inability of fetuses and pups to conjugate BPAF. Free and total concentrations in PND 28 pups were similar to LD 28 maternal concentrations, demonstrating direct exposure of pups via feed and indicating that conjugating enzymes are developed in PND 28 pups.

Genetic Toxicology

BPAF was not mutagenic in tests conducted with three strains of bacteria, with and without induced rat liver S9 mix. BPAF was also evaluated in the *in vivo* peripheral blood micronucleus assay for its ability to induce chromosomal damage in the form of structural or numerical alterations. No significant increases in the frequencies of micronucleated immature erythrocytes

(PCEs) were observed in male or female rats, and no significant changes in % PCE were observed, suggesting that BPAF exposure did not affect erythropoiesis.

Conclusions

Under the conditions of this modified one-generation (MOG) study, there was *clear evidence of reproductive toxicity* of bisphenol AF (BPAF) in Hsd:Sprague Dawley[®] SD[®] rats based on the increased disruption of estrous cyclicity, the inability of the F₁ generation to reproduce, decreases in F₁ pup survival, and a slight increase in gestation length for F₀ females at the highest dietary exposure concentration and, at lower concentrations, decreases in the number of implants, corpora lutea, and live fetuses or litters.

Under the conditions of this MOG study, there was *clear evidence of developmental toxicity* of BPAF in Hsd:Sprague Dawley[®] SD[®] rats based on the presence of fetal malformations and abnormal histopathology of both the male and female reproductive tract in the F₁ generation, impacts on developmental markers, including accelerated vaginal opening and delayed balanopreputial separation, and lower F₁ and F₂ mean body and organ weights.

Synonyms: 4,4'-(hexafluoroisopropylidene)diphenol; 2,2-bis(4-hydroxyphenyl)hexafluoropropane; 2,2-bis(4-hydroxyphenyl)perfluoropropane; phenol, 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]bis-; hexafluorobisphenol a

Bisphenol AF, NTP DART 08

Summary of Exposure-related Findings in Rats in the Modified One-Generation Study of Bisphenol AF

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
F₀ Generation				
Maternal Parameters				
Number mated	35	35	35	35
Number pregnant (%)	30 (85.7)	32 (91.4)	33 (94.3)	29 (82.9)
Number not pregnant (%)	5 (14.3)	3 (8.6)	2 (5.7)	6 (17.1)
Number littered (%) ^a	25 (92.6)	28 (96.6)	29 (96.7)	25 (96.2)
Gestation length (days)	22.1 ± 0.1**	22.2 ± 0.1	22.1 ± 0.1	22.4 ± 0.1**
Clinical Observations				
	None	None	None	None
Mean Body Weight and Feed Consumption^{b,c}				
Body weight: GD 21	356.8 ± 7.6**	359.7 ± 5.9	333.2 ± 3.8**	309.9 ± 4.4**
Body weight gain: GD 6–21	119.3 ± 7.0**	120.6 ± 4.2	94.8 ± 2.9**	71.9 ± 4.1**
Feed consumption: GD 6–21	20.0 ± 0.3*	20.5 ± 0.4	19.5 ± 0.7	19.2 ± 0.5
Body weight: LD 28	279.9 ± 3.3	273.7 ± 3.1	270.3 ± 3.0	274.4 ± 3.0
Body weight gain: LD 4–28	1.7 ± 2.5**	-1.6 ± 2.6	25.4 ± 2.5**	41.6 ± 2.8**
Feed consumption: LD 1–13	49.0 ± 0.7	47.6 ± 1.1	50.7 ± 1.4	54.4 ± 2.3
Necropsy Observations				
	None	None	None	None
F₁ Generation (Prewaning)^c				
Clinical Observations				
	None	None	None	Yellow fur
Live Litter Size				
PND 0	13.2 ± 0.4	11.9 ± 0.6	12.9 ± 0.4	12.5 ± 0.5
PND 1	13.0 ± 0.4	11.4 ± 0.7	12.6 ± 0.4	11.4 ± 0.7
PND 4 (prestandardization)	13.1 ± 0.4*	11.6 ± 0.6	12.4 ± 0.4	10.6 ± 0.8**
PND 4 (poststandardization)	9.8 ± 0.2	9.5 ± 0.3	10.0 ± 0.0	9.0 ± 0.5
PND 28	9.7 ± 0.2	9.0 ± 0.4	9.2 ± 0.3	8.6 ± 0.4
Male Pup Mean Body Weight				
Body weight: PND 1	6.82 ± 0.07**	6.58 ± 0.17	6.18 ± 0.12**	5.84 ± 0.19**
Body weight: PND 28	77.54 ± 1.30**	76.34 ± 1.38	68.49 ± 1.31**	53.94 ± 1.48**
Body weight gain: PND 4–28	67.77 ± 1.16**	66.60 ± 1.29	59.79 ± 1.18**	45.82 ± 1.32**
Female Pup Mean Body Weight				
Body weight: PND 1	6.57 ± 0.07**	6.34 ± 0.12	5.98 ± 0.11**	5.56 ± 0.12**
Body weight: PND 28	71.32 ± 1.34**	69.14 ± 1.13	64.65 ± 1.31**	51.92 ± 1.33**
Body weight gain: PND 4–28	62.05 ± 1.21**	59.93 ± 1.00	56.09 ± 1.18**	44.15 ± 1.18**
F₁ Generation (Postweaning)				
Mean Body Weight and Feed Consumption^{b,c}				
Male body weight: PND 28	76.3 ± 1.4**	75.5 ± 1.5	67.6 ± 1.4**	53.6 ± 1.6**
Male body weight: PND 98	386.3 ± 4.4**	373.7 ± 5.2	334.4 ± 5.0**	238.3 ± 4.6**
Male body weight gain: PND 28–91	299.7 ± 3.0**	286.9 ± 4.4	257.8 ± 4.1**	177.5 ± 4.0**
Male feed consumption: PND 28–98	21.8 ± 0.2**	21.3 ± 0.4	19.8 ± 0.3**	18.5 ± 0.3**
Female body weight: PND 28	69.5 ± 1.4**	69.3 ± 1.3	63.5 ± 1.6**	51.5 ± 1.4**
Female body weight: PND 98	242.9 ± 3.4**	227.2 ± 3.7**	206.0 ± 2.3**	172.6 ± 2.3**
Female body weight gain: PND 28–98	173.3 ± 2.9**	157.8 ± 3.1**	142.6 ± 2.1**	121.2 ± 1.7**
Female feed consumption: PND 28–98	15.6 ± 0.2**	16.3 ± 0.4	15.6 ± 0.4	14.5 ± 0.4*
F₁ and F₂ Generations				
Endocrine Endpoints, Developmental Landmarks, and Pubertal Endpoints^c				
Vaginal opening (F ₁)				
Adjusted mean day of vaginal opening (litter mean) ^d	35.8 ± 0.3**	33.8 ± 0.3**	27.8 ± 0.3**	27.9 ± 0.7**
Body weight at acquisition ^b	103.2 ± 1.7**	90.9 ± 1.6**	63.2 ± 1.4**	60.5 ± 2.6**

Bisphenol AF, NTP DART 08

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Balanopreputial separation (F₁)				
Adjusted mean day of balanopreputial separation (litter mean) ^d	46.4 ± 0.2**	46 ± 0.3	50.8 ± 0.7**	78.3 ± 2.1**
Body weight at acquisition ^b	200.4 ± 1.8**	188.0 ± 2.1**	195.1 ± 3.5	217.9 ± 3.6**
Number not attaining	0	0	0	10 (9)
Testicular descent (F₁)				
Mean day of testes descent (litter mean)	18.0 ± 0.2	17.9 ± 0.2	17.7 ± 0.3	18.1 ± 0.5
Number not attaining	0	0	1 (1)	11 (7)
Vaginal opening (F₂)				
Adjusted mean day of vaginal opening (litter mean) ^d	34.7 ± 0.3**	31.3 ± 0.6**	25.1 ± 0.5**	— ^e
Body weight at acquisition ^b	113.8 ± 1.8**	94.3 ± 2.5**	65.9 ± 2.2**	—
Balanopreputial separation (F₂)				
Adjusted mean day of balanopreputial separation (litter mean) ^d	46.5 ± 0.7**	45.0 ± 0.4	52.1 ± 1.1**	—
Body weight at acquisition ^b	209.5 ± 4.4	195.1 ± 2.6*	222.1 ± 7.4	—
Testicular descent (F₂)				
Mean day of testes descent (litter mean)	15.8 ± 0.4**	16.4 ± 0.3	17.7 ± 0.3*	—
Number not attaining	1 (1)	0	0	—
Prenatal Cohort				
Mating and Fertility Performance				
Number of mating pairs	21	21	22	19
Mated females/paired (%)	81.0**	95.2	86.4	0.0**
Pregnant females/mated (%)	100.0	100.0	94.7	—
Mean Body Weight and Feed Consumption^{b,c}				
Body weight gain: GD 0–21	169.2 ± 2.7**	143.9 ± 4.4**	90.4 ± 9.1**	—
Feed consumption: GD 0–21	22.7 ± 0.3**	21.6 ± 0.5	19.7 ± 0.5**	—
Uterine Content Data^c				
Mean number of corpora lutea/female	15.82 ± 0.44**	14.20 ± 0.47**	11.89 ± 0.52**	—
Implantations/female	15.00 ± 0.37**	13.85 ± 0.39*	8.73 ± 0.69**	—
Live fetuses/litter	14.63 ± 0.34	13.25 ± 0.52	7.29 ± 1.06**	—
Fetal weight/litter	5.09 ± 0.07**	4.98 ± 0.06	3.81 ± 0.35**	—
Fetal Findings				
External findings	None	None	None	—
Visceral findings	None	None	None	—
Head findings^f				
Dilated lateral ventricle, bilateral – [V]				
Fetuses	0 (0.00)	0 (0.00)	4 (8.33)	—
Litters	0 (0.00)	0 (0.00)	4 (30.77)	—
Misshapen lateral ventricle, left – [V]				
Fetuses	0 (0.00)	0 (0.00)	1 (2.08)	—
Litters	0 (0.00)	0 (0.00)	1 (7.69)	—
Skeletal findings^f				
Lumbar, 1, rudimentary, total – [V]				
Fetuses	11 (4.70)	19 (7.17)	14 (13.73)	—
Litters	6 (37.50)	10 (50.00)	4 (28.57)	—
Lumbar, 1, full, total – [M]				
Fetuses	0 (0.00)	4 (1.51)	0 (0.00)	—
Litters	0 (0.00)	3 (15.00)	0 (0.00)	—

Bisphenol AF, NTP DART 08

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Reproductive Performance Cohort				
Mating and Fertility Performance				
Number of mating pairs	22	23	21	19
Mated females/paired (%)	100.0**	100.0	76.2*	5.3**
Pregnant females/mated (%)	81.8*	95.7	75.0	0.0
Littered females/mated (%)	81.8**	87.0	56.3	0.0
Mean Body Weight and Feed Consumption^{b,c}				
Body weight gain: GD 0–21	158.0 ± 6.1**	132.8 ± 6.8*	95.3 ± 10.7**	–
Feed consumption: GD 0–21	23.5 ± 0.4**	22.3 ± 0.7	20.1 ± 1.0**	–
Body weight: LD 28	305.4 ± 3.7**	281.1 ± 3.8**	264.6 ± 6.9**	–
Body weight gain: LD 4–28	–8.5 ± 2.8**	–7.7 ± 2.3	16.1 ± 4.8**	–
Feed consumption: LD 1–13	44.9 ± 1.6	45.8 ± 0.9	37.0 ± 4.0	–
Live Litter Size^c				
PND 0	11.2 ± 1.0*	10.6 ± 0.8	6.4 ± 1.4*	–
PND 4 (prestandardization)	10.9 ± 1.0*	11.1 ± 0.6	6.4 ± 1.4*	–
PND 4 (poststandardization)	7.3 ± 0.4	7.9 ± 0.1	5.7 ± 1.1	–
PND 28	7.2 ± 0.4	7.7 ± 0.1	5.2 ± 1.1	–
Male Pup Mean Body Weight (Prewaning)^{b,c}				
Body weight: PND 1	7.32 ± 0.15	7.00 ± 0.16	6.80 ± 0.25	–
Body weight: PND 28	88.86 ± 2.01**	86.96 ± 1.49	77.82 ± 4.07*	–
Body weight gain: PND 4–28	77.63 ± 1.64**	76.12 ± 1.20	67.07 ± 3.63**	–
Female Pup Mean Body Weight (Prewaning)^{b,c}				
Body weight: PND 1	7.15 ± 0.15**	6.79 ± 0.14	6.28 ± 0.32*	–
Body weight: PND 28	81.62 ± 1.31**	78.23 ± 1.15	71.69 ± 2.52**	–
Body weight gain: PND 4–28	70.82 ± 1.09**	67.83 ± 0.94	62.17 ± 1.98**	–
Mean Body Weight and Feed Consumption (Postweaning)^{b,c}				
Male body weight: PND 28	87.5 ± 2.5*	85.8 ± 1.6	78.6 ± 4.1	–
Male body weight: PND 91	387.9 ± 6.7*	372.4 ± 5.3	360.2 ± 9.8	–
Male body weight gain: PND 28–91	300.4 ± 5.8	286.6 ± 4.5	281.6 ± 7.3	–
Male feed consumption: PND 28–91	22.0 ± 0.2**	21.5 ± 0.2	20.7 ± 0.4**	–
Female body weight: PND 28	81.1 ± 1.7**	76.8 ± 1.2	73.9 ± 1.9*	–
Female body weight: PND 91	240.3 ± 4.2**	217.6 ± 4.0**	203.9 ± 5.9**	–
Female body weight gain: PND 28–91	159.2 ± 3.6**	140.8 ± 3.9**	130.0 ± 5.9**	–
Female feed consumption: PND 28–91	16.0 ± 0.2*	15.4 ± 0.3	14.8 ± 0.6	–
Adult Necropsies				
Clinical Pathology (Subchronic Cohort)				
Hematology				
Male	NA	None	None	None
Female	NA	None	None	↓ Erythrocytes, hemoglobin, total white blood cells
Clinical chemistry				
Male	NA	None	↓ Cholesterol, bile acids	↓ Cholesterol, bile acids
Female	NA	↓ Cholesterol	↓ Cholesterol	↓ Cholesterol ↑ Triglycerides
Gross Necropsy Findings				
Prenatal cohort				
Male				
Cowper's gland				
Missing, left ^e	0	0	1 (1)	0

Bisphenol AF, NTP DART 08

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Missing, bilateral	0*	0	0	2 (2)
Missing, total	0	0	1 (1)	2 (2)
Size, reduced, bilateral	0**	0	0	14 (14)**
LABC				
Size, reduced	0**	0	0	18 (18)**
Prostate gland				
Size, reduced	0**	0	0	20 (20)**
Seminal vesicles				
Size, reduced, left	0	0	1 (1)	0
Size, reduced, right	0	1 (1)	0	0
Size, reduced, bilateral	0**	0	0	20 (20)**
Size, reduced, total	0**	1 (1)	1 (1)	20 (20)**
Female				
Ovaries				
Size, reduced, left	0*	0	0	2 (2)
Size, reduced, right	0*	0	0	2 (2)
Size, reduced, bilateral	0**	0	1 (1)	17 (17)**
Size, reduced, total	0**	0	1 (1)	19 (19)**
Uterus				
Size, reduced, bilateral	0**	0	1 (1)	19 (19)**
Vagina				
Misshapen	0	0	0	1 (1)
Reproductive performance cohort				
Male				
Cowper's gland				
Missing, bilateral	0*	0	0	2 (2)
Size, reduced, left	0	1 (1)	0	0
Size, reduced, bilateral	0**	1 (1)	0	14 (14)**
Size, reduced, total	0**	2 (2)	0	14 (14)**
LABC				
Size, reduced	0**	0	0	16 (16)**
Dorsolateral prostate gland				
Size, reduced	0**	0	0	18 (18)**
Ventral prostate gland				
Size, reduced	0**	0	0	18 (18)**
Seminal vesicles				
Size, reduced, bilateral	0**	0	0	18 (18)**
Phallus				
Misshapen	0	0	0	1 (1)
Female				
Ovaries				
Size, reduced, bilateral	0**	0	0	18 (18)**
Vagina				
Deformity	0	0	0	1 (1)
Misshapen	0	0	0	1 (1)
Subchronic cohort				
Male				
Prostate gland				
Size, reduced	0**	0	0	10 (10)**
Seminal vesicles				
Size, reduced, bilateral	0**	0	0	10 (10)**

Bisphenol AF, NTP DART 08

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Phallus				
Deformity	0	0	0	1 (1)
Female				
Ovaries				
Size, reduced, bilateral	0**	0	0	9 (9)**
Uterus				
Size, reduced, bilateral	0**	0	0	9 (9)**
F ₂ pups				
Male				
Cowper's gland				
Size, reduced, left	1 (1)	0	0	–
Size, reduced, bilateral	1 (1)	0	3 (3)	–
Size, reduced, total	2 (2)	0	3 (3)	–
LABC				
Size, reduced	0	0	2 (2)	–
Dorsolateral prostate gland				
Size, reduced	1 (1)	0	4 (3)	–
Ventral prostate gland				
Size, reduced	1 (1)*	0	5 (3)	–
Seminal vesicles				
Size, reduced, bilateral	0*	0	5 (3)	–
Organ Weights				
Prenatal cohort				
Male	NA	None	↓ Absolute dorsolateral prostate gland weight ↓ Absolute and relative ventral prostate gland weights ↓ Absolute seminal vesicles with coagulating gland weight	↓ Absolute and relative dorsolateral prostate gland weights ↓ Absolute and relative ventral prostate gland weights ↓ Absolute and relative seminal vesicles with coagulating gland weights ↓ Absolute and relative LABC weights ↓ Absolute and relative Cowper's gland weights
Female	NA	None	↓ Absolute right ovary weight ↓ Absolute left ovary weight	–
Reproductive performance cohort				
Male	NA	None	↓ Absolute ventral prostate gland weight ↓ Absolute seminal vesicles with coagulating gland weight	↓ Absolute and relative dorsolateral prostate gland weights ↓ Absolute and relative ventral prostate gland weight

Bisphenol AF, NTP DART 08

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Female	NA	None	↓ Absolute and relative right ovary weights ↓ Absolute and relative left ovary weights	prostate gland weights ↓ Absolute and relative seminal vesicles with coagulating gland weights ↓ Absolute and relative LABC weights ↓ Absolute and relative Cowper's gland weights –
Subchronic cohort Male	NA	None	↓ Absolute and relative ventral prostate gland weights	↑ Relative adrenal glands weight ↑ Relative thyroid gland weight ↑ Relative lung weight ↓ Absolute lung weight ↓ Absolute and relative liver weights ↓ Absolute and relative left kidney weights ↓ Absolute and relative dorsolateral prostate gland weights ↓ Absolute and relative ventral prostate gland weights ↓ Absolute and relative seminal vesicles with coagulating gland weights
Female	NA	None	↓ Absolute right ovary weight ↓ Absolute and relative left ovary weights ↓ Absolute uterus/cervix/vaginal weight	↓ Absolute and relative right ovary weights ↓ Absolute and relative left ovary weights ↓ Absolute uterus/cervix/vaginal weight

Bisphenol AF, NTP DART 08

		0 ppm	338 ppm	1,125 ppm	3,750 ppm
					↑ Relative thyroid gland weight ↑ Relative liver weight
F ₂ pups					
Male	NA		↓ Absolute and relative dorsolateral prostate gland weights ↓ Absolute seminal vesicles with coagulating gland weight ↓ Absolute and relative Cowper's gland weights ↓ Absolute LABC weight	↓ Absolute and relative dorsolateral prostate gland weights ↓ Absolute and relative ventral prostate gland weights ↓ Absolute and relative seminal vesicles with coagulating gland weights ↓ Absolute and relative Cowper's gland weights ↓ Absolute and relative LABC weights	–
Female	NA		↓ Absolute right ovary weight ↓ Absolute left ovary weight	↓ Absolute and relative right ovary weights ↓ Absolute and relative left ovary weights	–
Nonneoplastic Lesions					
Reproductive performance cohort					
Male					
Prostate gland					
		0**	0	0	18 (18)**
		0**	0	0	18 (18)**
Seminal vesicles					
		0**	0	0	18 (18)**
Coagulating gland					
		0**	0	0	18 (18)**
Cowper's gland					
		0**	0	0	15 (15)**
		0	1 (1)	0	0
		0**	1 (1)	0	15 (15)**
LABC					
		0**	0	1 (1)	17 (17)**
Testis					
		0**	0	1 (1)	6 (6)**
		0**	0	0	11 (11)**
		0**	0	0	8 (8)**

Bisphenol AF, NTP DART 08

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Epididymis				
Duct, atrophy	0**	0	0	10 (10)**
Duct, hypospermia	0**	0	1 (1)	6 (6)**
Duct, exfoliated germ cell	0**	0	1 (1)	5 (5)*
Female				
Ovary				
Hypoplasia, bilateral	0**	1 (1)	0	20 (20)**
Hypoplasia, unilateral	0	2 (2)	0	0
Hypoplasia, total	0**	3 (3)	0	20 (20)**
Uterus				
Hypoplasia	0**	0	0	18 (18)**
Epithelial, metaplasia, squamous	0**	0	0	20 (20)**
Dilation, glandular, cystic	0**	0	0	8 (8)**
Stroma, hyalinization	0**	0	8 (8)**	18 (18)**
Subchronic cohort				
Male				
Prostate gland				
Hypoplasia, dorsolateral	0**	0	0	10 (10)**
Hypoplasia, ventral	0**	0	0	10 (10)**
Seminal vesicles				
Hypoplasia, bilateral	0**	0	0	10 (10)**
Female				
Ovary				
Hypoplasia, bilateral	0**	0	0	10 (10)**
Uterus				
Hypoplasia	0**	0	0	10 (10)**
Epithelial, metaplasia, squamous	0**	0	0	10 (10)**
Dilation, glandular, cystic	0**	0	0	6 (6)**
Stroma, hyalinization	0**	0	0	10 (10)**
Andrology^c				
F ₁ males				
Left cauda epididymis weight	0.262 ± 0.004**	0.249 ± 0.004	0.222 ± 0.006**	0.130 ± 0.007**
Left testis weight	2.039 ± 0.026**	1.965 ± 0.028	1.876 ± 0.047**	1.469 ± 0.057**
Sperm (10 ⁶ /g cauda epididymis)	843.4 ± 27.3**	835.2 ± 26.3	796.9 ± 38.3	704.1 ± 27.1**
Spermatid heads (10 ⁶ /g testis)	120.9 ± 3.9**	128.5 ± 3.5	128.0 ± 3.9	148.8 ± 6.3**
F ₂ males				
Left cauda epididymis weight	0.211 ± 0.005**	0.198 ± 0.003**	0.171 ± 0.005**	—
Left testis weight	2.014 ± 0.023**	1.855 ± 0.026**	1.851 ± 0.041**	—
Vaginal Cytology				
F ₁ females	NA	None	↑ Estrous cycle length	↓ Diestrus stage length ↑ Proestrus stage length ↑ Estrus stage length
F ₂ females	NA	↑ Estrous cycle length ↓ Proestrus stage length ↓ Estrus stage length	↑ Estrous cycle length	—

Bisphenol AF, NTP DART 08

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Level of Evidence of Reproductive Toxicity: Clear evidence				
Level of Evidence of Developmental Toxicity: Clear evidence				
Genetic Toxicology				
Bacterial mutagenicity				
<i>Salmonella typhimurium</i> strains TA98 and TA100		Negative		
<i>Escherichia coli</i> strain WP2 <i>uvrA</i> (pKM101)		Negative		
Peripheral blood micronucleus assay				
Male and female Sprague Dawley rats		Negative		

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

GD = gestation day; LD = lactation day; PND = postnatal day; [V] = variation; [M] = malformation; NA = not applicable; LABC = levator ani/bulbocavernosus muscle.

^aThree F₀ dams were removed in each exposure group on GD 18 for biological sample collection.

^bBody weight results given in grams. Feed consumption results given in grams/animal/day.

^cData are presented as mean \pm standard error.

^dAdjusted based on body weight at weaning.

^eNo F₁ females were confirmed pregnant for the 3,750 ppm group.

^fUpper row denotes number of affected fetuses (%) and lower row the number of affected litters (%).

^gNumber of animals (number of litters) with lesion.

Introduction

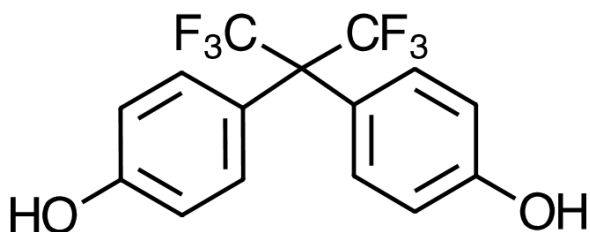


Figure 1. Bisphenol AF (CASRN 1478-61-1; Chemical Formula: C₁₅H₁₀F₆O₂; Molecular Weight: 336.23)

Synonyms: 4,4'-(hexafluoroisopropylidene)diphenol; 2,2-bis(4-hydroxyphenyl)hexafluoropropane; 2,2-bis(4-hydroxyphenyl)perfluoropropane; phenol, 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]bis-; hexafluorobisphenol a.

Chemical and Physical Properties

Bisphenol AF (BPAF) is a white to light gray powder with a melting point of 160°C–162°C. BPAF is relatively insoluble in water (estimated median of 209 ppm at 21.5°C; log octanol/water partition coefficient [K_{ow}] = 4.64 ± 0.10) and is soluble in most organic solvents.^{1;2}

Production, Use, and Human Exposure

BPAF can be synthesized from the reaction of a phenol and a fluorinated precursor (e.g., fluorinated aldehyde or ketone) in the presence of an organic sulfonic acid catalyst.³

BPAF is commonly used as a curing or crosslinking agent in the processing of fluorocarbon elastomers⁴ and in rubber processing to alter polymer properties of a compound by forming covalent, hydrogen, or other bonds between polymer molecules.⁵ BPAF is often used in combination with triphenyl benzylphosphonium chloride (BTTPC/BeTPC) in organic synthesis as a wetting reagent and as a phase transfer catalyst in the production of fluoroelastomers and printing inks. BPAF-cured fluoroelastomers have material characteristics, including thermal stability, chemical resistance, and compression set resistance, which are useful in plastics manufacturing and other fabrication processes. In addition, BPAF is used as a monomer in the synthesis of other specialty polymers, including polyimides, polyamides, polyesters, and polycarbonates, allowing use in a wide range of specialty applications (e.g., in gas separation and semiconductor processing).⁶ Polymers containing BPAF are useful in high-temperature composites, electronic materials, and other specialty applications.

BPAF's physical and chemical properties suggest that it resides primarily in soil, water, or sediment, depending on the mode of entry, and that it is not readily biodegradable.⁷ Predicted half-life in atmospheric conditions (0.133 days) suggests that BPAF (neutral form) is not persistent in air; however, biodegradation models indicate that the half-life in water/soil and sediment is >182 days and 365 days, respectively.⁷

BPAF has been detected in the general population around the world, suggesting environmental exposure. Free BPAF was detected in 85% of urine samples from university students in South

China, with a median urine concentration of 0.03 ng/mL.⁸ The frequency of detection of BPAF reported in other studies in China, however, was <6.5%.^{9; 10} Urinary concentrations of total BPAF were also low (≤ 0.173 ng/mL) and were found in <30% of samples tested.⁹ BPAF was below the limit of detection (LOD) of 0.02 μ g/mL in urine samples from a European Union biomonitoring study of 144 adults.¹¹ Urinary BPAF concentrations up to 88.3 ng/L were observed in children, but the mean concentration was only 3.4 ng/L due to the low detection rate of 46%.¹²

In serum, BPAF was detected in 33% of samples from 181 pregnant women in China with a concentration up to 0.4 ng/mL.¹³ It was also detected in 100% of maternal plasma, cord plasma, and placenta samples from 60 women in South China,¹⁴ with mean BPAF concentrations of 13.1, 80.4, and 28.4 pg/g, respectively. The higher concentrations in cord plasma relative to maternal plasma and placenta suggest a high maternal transfer rate and the potential for accumulation in fetal cord blood. BPAF was also detected in 21% of human breast milk samples with a mean concentration of 0.092 ng/mL.¹⁵ In serum from an elderly population near an e-waste recycling plant, BPAF concentrations up to 0.043 ng/mL were reported.¹⁶

Regulatory Status

BPAF is part of the compiled inventory of substances likely to meet the criteria of Annex III to the European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation, and it is listed as both a suspected hazard to the aquatic environment and as persistent in the environment. In the United States, it is listed in the Toxic Substances Control Act (TSCA) inventory and, in its dipotassium salt form (phenol, 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]bis-, dipotassium salt (CASRN 25088-69-1)), is approved as an indirect food additive (Section 177.2400 perfluorocarbon-cured elastomers) and can be used as articles or components of articles intended for repeated use in contact with nonacid food (pH above 5.0).^{17; 18}

Absorption, Distribution, Metabolism, Excretion, and Toxicokinetics

Experimental Animals

The National Toxicology Program (NTP) investigated the absorption, distribution, metabolism, and excretion (ADME) and toxicokinetic properties of BPAF in male and female Sprague Dawley (Hsd:Sprague Dawley[®] SD[®]) rats and B6C3F1/N mice.^{19; 20} BPAF was excreted primarily in feces following a single gavage dose of 3.4, 34, or 340 mg/kg body weight [¹⁴C]-labeled BPAF administered to rats (65%–80%) or to mice (63%–72%).¹⁹ Excretion of [¹⁴C]-labeled BPAF in urine was low to moderate, with females (rat, 15%; mouse, 24%) excreting more than males (rat, 4%; mouse, 10%) following a single 34 mg/kg gavage dose. Concomitant with higher urinary excretion, the fecal excretion was lower in females (rat, 65%; mouse, 53%) compared to males (rat, 77%; mouse, 72%).¹⁹ Approximately 52% of an orally administered 34 mg/kg dose in male rats was recovered in bile 24 hours postadministration. Most of the dose excreted in feces, however, was recovered after 24 hours; this delay in fecal excretion supports enterohepatic recirculation of BPAF-derived moieties.¹⁹ Following a 34 mg/kg intravenous dose in male and female rats and mice, 63%–76% of the administered dose was recovered in feces

with $\leq 16\%$ recovered in urine.¹⁹ Taken collectively, these data demonstrate that BPAF was well-absorbed in rats and mice after gavage administration and that most of the dose recovered in feces of rats and mice (63%–80%) was likely the absorbed dose, which was excreted via bile to the intestine. [¹⁴C]-labeled BPAF was distributed to tissues, but the total radioactivity in tissues at 72 hours postadministration was $\leq 2\%$, showing low potential for tissue retention.¹⁹

Metabolites identified in bile were monoglucuronide, diglucuronide, and mixed glucuronide sulfate conjugates of the [¹⁴C]-labeled BPAF; parent BPAF was not detected.¹⁹ The main analyte detected in feces was the parent BPAF, however, which suggests deconjugation of metabolites in the intestine before excretion via feces. In urine, parent [¹⁴C]-labeled BPAF and mono- and diglucuronides were detected. The metabolism of BPAF in rodents is shown in Figure 2.¹⁹ Li et al.²¹ also reported four urinary metabolites of BPAF (diglucuronide, glucuronide, a dehydrated form of glucuronide, and a sulfate conjugate) after daily gavage administration of 200 mg/kg in Sprague Dawley rats for 2 weeks.

Toxicokinetic properties of both free BPAF (parent) and total BPAF (combined parent and conjugated forms) were investigated after a single oral dose of 34, 110, or 340 mg/kg in Hsd:Sprague Dawley[®] SD[®] rats and B6C3F1/N mice.²⁰ BPAF was absorbed rapidly in male and female rats with a time to reach maximum concentration (T_{\max}) of ≤ 2.20 hours for free BPAF. BPAF was cleared rapidly with a plasma elimination half-life of ≤ 3.35 hours. The maximum concentration (C_{\max}) and the area under the concentration versus time curve (AUC) of free BPAF increased proportionally to the dose in both sexes. Total BPAF T_{\max} was also short in male and female rats (≤ 1.07 hours). In addition, total C_{\max} and AUC values were ≥ 27 -fold and ≥ 52 -fold higher, respectively, than corresponding free BPAF values. These data demonstrate rapid and extensive first pass conjugation of BPAF in the intestine and the liver after oral administration. Absorption of BPAF after oral administration of the 34 mg/kg dose in mice was more rapid than in rats, with the C_{\max} for free BPAF reached at 0.46 hours; however, plasma elimination of free BPAF was slightly slower than in rats, with a half-life ≤ 4.22 hours. As in rats, C_{\max} and AUC in mice were much higher for total BPAF than for free BPAF (≥ 30 -fold and ≥ 12 -fold higher, respectively). Consistent with this finding, the oral bioavailability in rats (approximately 1%) and mice (3%–6%) was low. There was no apparent sex-related effect in toxicokinetic parameters for free or total BPAF in rats or mice.²⁰

Following exposure of male Hsd:Sprague Dawley[®] SD[®] rats and B6C3F1/N mice to 338, 1,125, or 3,750 ppm BPAF via feed for 7 days, free BPAF C_{\max} and AUC, per a unit BPAF dose were four- to ninefold and two- to sixfold higher, respectively, in mice than in rats. The difference was greater at the higher exposure concentration likely due to lower chemical consumption by rats in the 3,750 ppm group. For total BPAF, rats showed a higher systemic exposure compared to mice at lower exposure concentrations. At the highest exposure concentration, the total BPAF systemic exposure was either lower (C_{\max}) or similar (AUC) to mice with the difference likely due to lower chemical consumption by rats in the highest exposure group (3,750 ppm). The total BPAF C_{\max} and AUC were higher than the corresponding free values in rats ($C_{\max} \geq 130$ -fold; AUC ≥ 127 -fold) compared to mice ($C_{\max} \geq 16$ -fold; AUC ≥ 16 -fold), demonstrating that the extent of conjugation of BPAF in rats was much higher than in mice. For free BPAF, the plasma elimination half-lives were similar between the two species (rats, 7.10–10.5 hours; mice, 4.50–6.66 hours); however, for total BPAF, half-lives were about two- to threefold longer in rats (7.44–13.3 hours) compared to mice (3.49–4.18 hours).²²

BPAF was rapidly taken up by zebrafish, with equilibrium concentrations reached in 24–72 hours following whole-body exposure to 1–20 $\mu\text{g}/\text{L}$.²³ BPAF concentrations were higher in males (8.5–174 $\mu\text{g}/\text{kg}$) than in females (5.0–105 $\mu\text{g}/\text{kg}$) at all exposure concentrations after 168 hours of exposure. Glucuronide conjugate of BPAF was the major metabolite observed, and concentrations found were higher than those of free BPAF. Glucuronide concentrations were higher in females (approximately 54–1,180 $\mu\text{g}/\text{kg}$) than in males (approximately 24–533 $\mu\text{g}/\text{kg}$), which, combined with the lower free BPAF concentrations in females, suggests more extensive glucuronidation in female zebrafish.

BPAF was cleared more rapidly by rat hepatocytes compared to mouse hepatocytes in vitro (the half-life for rat was 6–13 minutes and for mouse was approximately 36–66 minutes).¹⁹

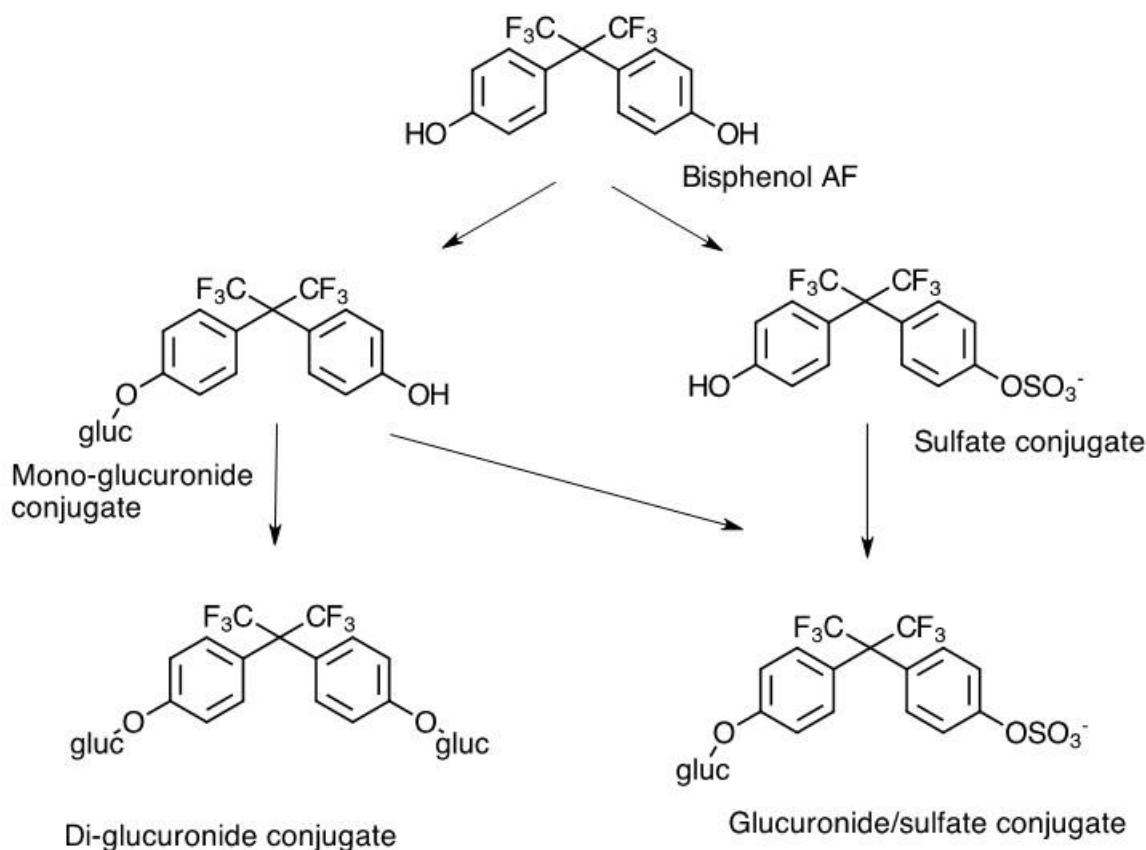


Figure 2. Metabolism of Bisphenol AF in Rodents

Adapted from Waidyanatha et al.¹⁹

Humans

The literature contains no studies on in vivo ADME data of BPAF in humans. In human hepatocytes in vitro, BPAF was cleared more slowly than in hepatocytes from rats or mice, with a half-life of 101–156 minutes. Metabolites corresponding to mono- and diglucuronides and sulfate conjugates were detected.¹⁹ Metabolism of BPAF to its glucuronide was also shown in human liver microsomes with an estimated maximum velocity (V_{max}) of 11.6 nmol/min/mg.²¹ The authors also reported glucuronidation of BPAF could be mediated through several human recombinant UDP-glucuronosyltransferases (UGTs), including UGT1A1, UGT1A3, UGT1A8,

UGT1A9, UGT2B4, UGT2B7, UGT2B15, and UGT2B17, among which UGT2B7 showed the highest efficiency of glucuronidation among those tested.²¹

Developmental and Reproductive Toxicity

Models of Endocrine Activity

BPAF has been reported to bind strongly to estrogen receptor alpha (ER α) and estrogen receptor beta (ER β) over estrogen-related receptor (ERR) gamma. Receptor-binding activity was stronger for ER β than for ER α ; however, when assessed using a reporter gene assay, BPAF was a full agonist for ER α and almost completely inactive in stimulating the basal constitutive activity of ER β . BPAF acted as a strong antagonist against the activity of the endogenous ER β agonist 17beta-estradiol. Results from Matsushima's research group suggest that BPAF can function as an endocrine-disrupting chemical, acting as either an agonist or antagonist, and can perturb physiological processes mediated through ER α and/or ER β .²⁴ BPAF also can activate ER-regulated gene transcription²⁵ and stimulate estrogen-sensitive human breast cancer cell (MCF-7) proliferation.^{26; 27} In the Hershberger assay (antagonist mode), however, BPAF-exposed rats displayed an increase in the relative weight of the Cowper's glands and glans penis along with significantly decreased body weights (approximately 6%–20%).²⁸

Studies conducted according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 407 suggest that BPAF has estrogenic properties as demonstrated by Leydig cell atrophy and irregular estrous cycles.²⁹ Uterotrophic and Hershberger repeated dose tests showed significant increases in relative uterine weights and significant increases in glans penis weight, respectively.²⁸

Experimental Animals

In a combined repeated dose toxicity study with reproduction/developmental toxicity screening (OECD Test Guideline 422) performed in Sprague Dawley rats administered BPAF via oral gavage,³⁰ the female fertility index decreased with increasing dose, and at the high dose of 300 mg BPAF/kg body weight/day (mg/kg/day), no pregnancies were observed (11, 10, 8, and 0 pregnant females at 0, 30, 100, and 300 mg/kg/day, respectively). Furthermore, the number of corpora lutea and the number of implants were also reduced with increasing dose. No adverse effects on offspring or differences in sex ratio and body weights of offspring (up to postnatal day [PND] 4) were noted between treated and control animals. In the same study, effects on male reproductive organs were noted, and Leydig cell atrophy was observed at the 100 and 300 mg/kg/day doses.³⁰ This effect is consistent with effects reported for a 28-day repeated dose toxicity study²⁹ in which Leydig cell atrophy was observed in 5 out of 10 male Sprague Dawley rats exposed to 100 mg/kg/day (highest dose tested).

In an in vivo mammary gland study, CD-1 mice were exposed via oral gavage to 0, 0.05, 0.5, or 5 mg/kg BPAF twice per day from gestation day (GD) 10.5 to GD 17.5; offspring were observed for up to 16 months. BPAF exposure in this study resulted in accelerated pubertal mammary development and a significant dose-related increase in nonneoplastic lesions in BPAF-exposed groups by 14 months.³¹ Lactational transfer was observed in a cross-fostered study using Sprague Dawley rats in which BPAF was administered to dams by oral gavage during gestation (GD 3–19) or lactation (lactation days [LD] 3–19) at 0 and 100 mg/kg/day. Lactational exposure caused

significantly increased concentrations of BPAF in serum and testis from male rat pups, indicating that BPAF was transferred via breast milk.³²

Humans

The literature contains no studies on the developmental or reproductive toxicity of BPAF in humans.

General Toxicity

Experimental Animals

The rat oral median lethal dose (LD₅₀) has been reported to be 3,400 mg/kg.³³ Adverse effects have been observed in studies submitted to the European Chemicals Agency (ECHA), with a no-observed-adverse-effect level (NOAEL) of 10 mg/kg/day.³⁴

In a short-term repeat dose toxicity study in rats conducted according to OECD Test Guideline 407,²⁹ 10 animals/sex/group were administered 0, 10, 30, or 100 mg/kg/day BPAF via oral gavage for at least 28 days. In the 100 mg/kg/day group, salivation was noted early after dosing, although this sign disappeared within 90 minutes of administration. Primary findings included a decrease of body weight gains in males at 100 mg/kg/day and in females at both 30 and 100 mg/kg/day. In male rats, white blood cell counts, total cholesterol, and albumin values decreased in the 100 mg/kg/day group; in female rats, cholinesterase and total cholesterol values decreased and total bilirubin values increased in the 100 mg/kg/day group. Serum thyroxine (T₄) values increased in the 100 mg/kg/day groups of both sexes, but no changes in thyroid stimulating hormone (TSH) were detected in any dose groups. In male rats, relative kidney, adrenal, and brain weights increased significantly in the 100 mg/kg/day group, and the absolute prostate (ventral and dorsolateral), ventral prostate, seminal vesicle, liver, heart, and spleen weights decreased relative to the control group. In female rats, relative brain weight increased significantly in the 30 and 100 mg/kg/day groups, and absolute heart weight decreased in the 100 mg/kg/day group. In addition, pathological changes were noted in several tissues. The NOAEL for systemic toxicity was 10 mg/kg/day due to the reduction in body weight gain and abnormal estrous cycles in the female rats dosed at 30 mg/kg/day.

Humans

The literature contains no studies on the general toxicity of BPAF in humans.

Genetic Toxicity

The genetic toxicity of bisphenol A (BPA) and BPA analogs have been reviewed.³⁵ In contrast to some of the other BPA analogs, little information exists on the genotoxic potential of BPAF, although, of the studies available, several have published positive results for BPAF.

As part of a class study of BPA analogs, BPAF-induced cytotoxicity was measured in five different DT40 chicken lymphoblastoid cell lines, each deficient in a different key gene involved in DNA repair processes (e.g., nonhomologous end joining, base excision repair, translation synthesis, homologous recombination). In contrast to BPA and other BPA analogs, such as bisphenol M and bisphenol F (BPF), there was no evidence of enhanced cytotoxicity compared

with wild-type DNA repair competent DT40 cells observed in any of the five DT40 cell lines treated with BPAF (concentrations ≤ 25 μM).³⁶ Furthermore, BPAF did not induce chromosomal aberrations in any of the five DT40 knockout cell lines (Ku70^{-/-}, Pol β ^{-/-}, RAD54^{-/-}, REV3^{-/-}, and XPA^{-/-}), and no increase in γH2AX foci was observed in the single DT40 RAD54^{-/-} cell line tested, which had been shown to be most sensitive to DNA damage induced by BPA analogs.³⁶

In contrast to the DT40 studies, several studies in mammalian cell models reported positive results with BPAF. Hercog et al.³⁷ reported that BPAF, over a concentration range of 5–20 $\mu\text{g}/\text{mL}$, showed greater cytotoxicity than BPA, BPF, or bisphenol S in HepG2 cells after 24 and 72 hours of incubation. BPAF induced increases in γH2AX foci, indicators of DNA double strand breaks, at the 10 and 20 $\mu\text{g}/\text{mL}$ concentrations after 24 hours of exposure. Testing BPAF at a lower, environmentally relevant concentration (1 ng/mL), however, revealed no increases in γH2AX foci. Mixtures of the four BPA analogs at ng concentrations also did not result in increases in γH2AX foci.

In the *in vitro* alkaline comet assay using primary human peripheral lymphocytes, Mokra et al.³⁸ found significant increases in percent tail DNA at BPAF concentrations of 0.1–10 $\mu\text{g}/\text{mL}$ after 1 hour incubation. Significant increases in DNA damage also were observed in the neutral version of the comet assay at BPAF concentrations of 1 and 10 $\mu\text{g}/\text{mL}$ after 1 hour incubation, a response that the authors suggest is indicative of BPAF-induced DNA double strand breaks. Increased levels of DNA damage were also seen with BPAF in both the alkaline and the neutral comet assays following 4 hours incubation, in which the top dose was limited to 1 $\mu\text{g}/\text{mL}$. The observed increases in DNA damage induced by BPAF were greater than the increases seen with BPA, BPF, or bisphenol S over the same concentration ranges and incubation times.

Lei et al.³⁹ tested BPAF, BPA, bisphenol E, bisphenol C, tetrachlorobisphenol A, and thiodiphenol in the alkaline comet assay using MCF-7 cells over a concentration range of 1–50 μM in dimethyl sulfoxide. Increases in percent tail DNA were observed with BPAF and several of the other BPA analogs although only at doses that induced significant cytotoxicity, thus confounding interpretation of the results.

In a modified *in vitro* alkaline comet assay using DNA glycosylases designed to assess the relative amount of oxidative base damage induced by BPAF, BPA, BPF, and bisphenol S in primary human peripheral lymphocytes, BPAF was reported to significantly increase percent tail DNA at doses as low as 0.01 $\mu\text{g}/\text{mL}$ after 48 hours incubation and 0.1 $\mu\text{g}/\text{mL}$ after 4 hours incubation.⁴⁰ BPAF also induced greater increases in percent tail DNA than BPA and the other two analogs tested in the same assay.

Study Rationale

BPAF was selected for evaluation based on a review of compounds that are potentially endocrine-active after concerns were raised about possible effects of BPA on the brain, behavior, and prostate gland of fetuses, infants, and children at current human exposure levels. The review assessed a number of agents that could have endocrine activity and were either persistent in the environment or had high human exposures, including chemicals that are structurally related to BPA.

BPAF, a fluorinated analog of BPA used in the production of polycarbonates, fluoroelastomers, and epoxy resins, was therefore chosen due to the potential for endocrine activity, lack of

Bisphenol AF, NTP DART 08

adequate toxicity data, and possible environmental persistence due to the presence of fluorine atoms.

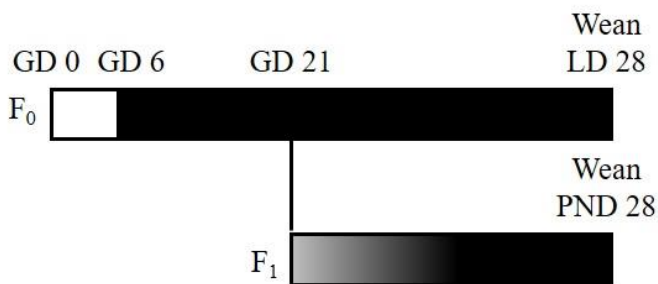
BPAF exposure via diet was selected for this study because the oral route is a relevant exposure pathway for humans. To minimize the potential endocrine activity of phytoestrogens, which are often present in rodent diets, a diet low in phytoestrogens was used.

Materials and Methods

Overview of Pre- and Postnatal Dose Range-finding and Modified One-Generation Study Designs

Modified one-generation (MOG) studies are composed of two interrelated parts: (1) a dose range-finding study (Figure 3) and (2) a MOG study (Table 1; Figure 4). If the acceptable range of exposure concentrations required to avoid excessive general and perinatal toxicity is unknown, a pre- and postnatal dose range-finding study is conducted. Nulliparous females are mated at the animal vendor and sent to the testing laboratory. Dosing typically begins at implantation (gestation day [GD] 6) and continues through weaning on lactation day (LD) 28. Offspring are exposed in utero, during lactation, and through consumption of dosed feed.

In MOG studies, time-mated females are administered the test article from GD 6 through weaning (evidence of mating = GD 0). The subsequent F₁ litters are standardized to a specified litter size (n = 8 or 10), with equal representation of both sexes. These offspring are continuously exposed to the test article via the same route of exposure and dose concentration as their dams. Multiple endpoints indicative of potential endocrine alteration (e.g., anogenital distance [AGD], nipple retention in males, pubertal markers) are measured (Table 1). Randomly selected F₁ animals are taken to adulthood for gross and histopathological examinations and can be allocated at weaning (postnatal day [PND] 28) to various cohorts. Histopathological examination of multiple animals per litter increases the power of statistical tests to detect adverse effects.⁴¹



Legend

- = No exposure
- = Exposure through feed
- = Exposure through lactation and then feed
- = Offspring

Figure 3. Design of a Dose Range-finding Study

F₀ dams are exposed to the test article from gestation day (GD) 6 through weaning on lactation day (LD) 28 and evaluated for maternal toxicity. F₁ offspring are exposed in utero through postnatal day (PND) 28 and evaluated for signs of in utero and postnatal toxicity.

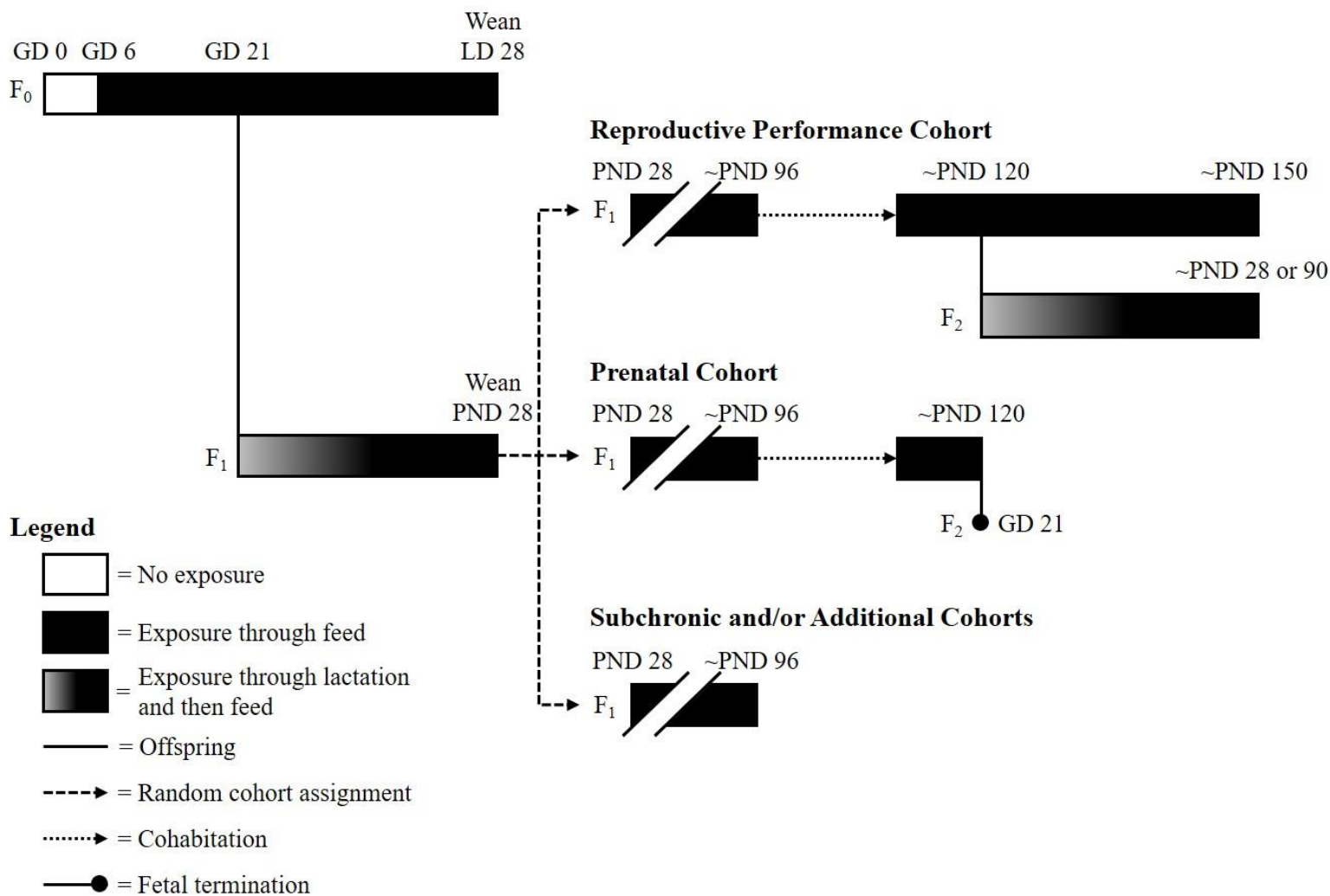


Figure 4. Design of a Modified One-Generation Rat Study

F₀ dams are exposed to the test article from gestation day (GD) 6 through weaning on lactation day (LD) 28 and evaluated for maternal toxicity. F₁ offspring are exposed in utero and during lactation through postnatal day (PND) 28 and evaluated for signs of toxicity. After weaning, F₁ offspring are allocated into cohorts for prenatal, reproductive performance, or additional assessments (e.g., subchronic or biological sampling cohorts) and exposure to test article continues until necropsy. F₂ offspring are exposed in utero and during lactation and postweaning until necropsy (reproductive performance cohort).

The ability of F₁ animals to mate and produce viable offspring is evaluated in the reproductive performance cohort. The potential for the test article to induce fetal defects is assessed in the prenatal cohort: F₂ fetuses are examined on GD 21, which includes examination of external morphology, fetal viscera, head (soft tissue and skeletal components), and skeleton (osseous and cartilaginous defects). Abnormalities are categorized as either malformations, which are permanent structural changes that could adversely affect survival, development, or function; or variations, which are a divergence beyond the usual range of structural constitution that might not adversely affect survival or health,⁴² consistent with descriptions by Makris et al.⁴³ Endpoints common to most cohorts are described in Table 1.

Table 1. Key Modified One-Generation Study Design Endpoints

Cohort	Key Endpoints
F₀ Dams	Maternal toxicity endpoints (body weight, feed consumption, clinical observations)
F₁ Generation^a	Clinical observations Body weights Feed consumption Necropsy Pup survival Anogenital distance, nipple/areola retention, testis descent, vaginal cytology
Reproductive Performance Cohort	F ₁ reproductive performance F ₁ andrology and sperm parameters F ₁ histopathology F ₂ litter size, viability, and growth F ₂ necropsy
Prenatal Cohort	F ₁ reproductive performance F ₂ fetal external, visceral, skeletal, and head soft tissue examinations F ₂ necropsy
Subchronic Cohort	F ₁ hematology F ₁ clinical chemistry F ₁ histopathology

^aAdditional cohorts (e.g., biological sampling cohort) and associated endpoints may be included in the study design.

Subchronic toxicity, including effects on clinical chemistry and hematology, are assessed in a 3-month cohort. Other cohorts can also be added (e.g., for internal dose estimation, neurobehavioral, toxicokinetic, and/or immunotoxicity assessments) to identify potential hazards across multiple functional outcomes. If necessary, more than one animal per sex can be selected from each litter and assigned to a cohort (e.g., reproductive performance). Examining multiple animals per litter increases the likelihood of detecting adverse responses and collectively makes the most use of the animals produced.

In the studies reported here, F₀ females were administered the test article in feed beginning on GD 6. F₁ and F₂ offspring were exposed in utero, during lactation, and through consumption of dosed feed.

Procurement and Characterization

BPAF was obtained from 3B Pharmachem International Co., Ltd (Wuhan, China) in a single lot (20100425) that was used in the dose range-finding and MOG studies. The bulk chemical of BPAF lot 20100425 was received in two batches, which were screened for identification and purity to ensure acceptable quality. Subsequently, the two batches were combined and homogenized by mixing for 5 minutes. The final batch was transferred to 80-oz amber glass bottles sealed with Teflon-lined lids and stored at ambient conditions. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at MRIGlobal (Kansas City, MO) (Appendix A). Reports on analyses performed in support of the BPAF studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot 20100425 of the chemical, a white powder, was identified as BPAF by infrared (IR), ¹H and ¹³C nuclear magnetic resonance (NMR), and ultraviolet/visible (UV/Vis) spectroscopies. The IR, ¹H, and ¹³C NMR spectra, and UV/Vis spectra (Appendix A) were consistent with reference spectra and the anticipated structure of BPAF. Direct infusion mass spectrometry (DIMS) confirmed the molecular weight. The melting point, octanol/water partition coefficient (K_{ow}), and elemental analysis of lot 20100425 matched BPAF.

The purity of lot 20100425 was determined using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection and differential scanning calorimetry (DSC). Headspace gas chromatography (GC/headspace) was performed to determine residual solvent content (Table A-1). Karl Fisher titration was conducted to estimate moisture content.

Purity assessment by HPLC/UV found one major peak accounting for 99.86% and one minor peak accounting for 0.13% of the total integrated area. Purity by DSC was 100%. No significant residual solvent impurities were found. Karl Fisher titration indicated a water content of 0.026%. The bulk purity of lot 20100425 was determined to be >99.5%.

Accelerated stability studies confirmed that lot 20100425 was stable for at least 2 weeks when stored in sealed glass vials at temperatures from -20°C to 60°C. Periodic reanalyses of the bulk chemical performed by the study laboratory using HPLC/UV showed no degradation.

Preparation and Analysis of Dose Formulations

Dose formulations of BPAF in LabDiet 5K96 Verified Casein Diet feed were prepared approximately monthly following the protocols outlined in Table A-2. Dose formulations of 0, 937.5, 1,875, 3,750, 7,500, and 15,000 ppm were used for the dose range-finding study, and dose formulations of 0, 338, 1,125, and 3,750 ppm were used for the MOG study. Formulations were stored in sealed plastic bag-lined containers for up to 42 days at 5°C.

The method of preparation was validated for concentration ranges of approximately 200–10,000 ppm. High-dose method verification confirmed that formulations up to approximately 45,000 ppm could be diluted into the validated calibration curve range. The optimal extraction solvent was determined to be acidified acetonitrile (99:1, acetonitrile:acetic acid, v:v).

Prior to study start, the stability and homogeneity of the formulations were determined using HPLC/UV. Stability of the 250 and 937.5 ppm formulations was confirmed for up to 42 days under refrigerated or freezer conditions while protected from light. A 7-day simulated dose study of the 250 and 937.5 ppm formulations was conducted to determine stability in animal room conditions. Formulations in the absence and presence of rodent urine and feces under dosing conditions were stable for up to 7 days. A decrease in recovery of BPAF was observed under simulated animal room conditions when the formulation was mixed with excreta, with recovery of BPAF reduced to approximately 77% by day 7, when compared to the day 0 determined concentration. However, when samples from the 7-day simulated dose study of the 937.5 ppm formulation spiked with rodent urine and feces were analyzed using an acid-digestion method, recovery increased to 90.8% on day 7. These results indicate extensive reversible binding of BPAF to feed in the presence of rodent urine and feces and not chemical instability when mixed with feed. Homogeneity of the dose formulations was confirmed at 250, 937.5, and 15,000 ppm in 22-kg preparations of dose formulations and at 338 and 3,750 ppm in 37-, 50-, and 100-kg preparations.

Analyses of pre- and postadministration dose formulations were conducted throughout the study using HPLC/UV to determine purity (Table A-3, Table A-4). All preadministration samples were within 10% of the target concentrations. Postadministration samples were collected from the animal room at the end of the exposure period. For the dose range-finding study, postadministration samples were within 30% of the target concentrations and within 28% of the preadministration concentrations. For the MOG study, postadministration samples were within 22% of the target concentrations and within 20% of the preadministration concentrations. Excreta contained in the samples might have affected BPAF recovery of the dose formulations as the results mimic findings from the 7-day simulated dose study. The concentration values for the postadministration samples were considered to have demonstrated acceptable stability.

Animal Source

Female Sprague Dawley (Hsd:Sprague Dawley[®] SD[®]) rats were obtained from Envigo (formerly Harlan Laboratories, Inc., Dublin, VA) for use in the dose range-finding and MOG studies. Sexually mature (11 to 12 weeks old) females were time-mated overnight at the vendor and were received on GD 1 or 2 for both the dose range-finding and MOG studies. GD 0 was defined as the day positive evidence of mating was observed.

Animal Health Surveillance

In accordance with the National Toxicology Program (NTP) Sentinel Animal Program (Appendix C), 10 female sentinel animals were evaluated in the dose range-finding study. Twenty female sentinel animals, as well as 15 F₁ male sentinel animals that were reassigned from the control group at weaning, were evaluated in the main study. All test results were negative.

Animal Welfare

Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. All animal studies were conducted in a facility accredited by AAALAC International. Studies were approved by the RTI International Animal Care and Use

Committee and conducted in accordance with all relevant National Institutes of Health and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Experimental Design

Dose Range-finding Study

Time-mated female rats were received on GDs 1 or 2, randomized based on GD 3 body weight, and placed on a 5K96 Casein diet containing 0, 937.5, 1,875, 3,750, 7,500, or 15,000 ppm BPAF from GD 6 through LD 28. Feed and water were available ad libitum. Information on feed composition and contaminants is provided in Appendix B. Dose selection was based on results of previously conducted studies in which BPAF was orally administered to rats.²⁸ No mortality was observed in rats administered up to 600 mg BPAF/kg body weight/day (mg/kg/day) by oral gavage for 3 days; therefore, a feed concentration of 15,000 ppm BPAF, estimated to be equivalent to a dose of 1,000 mg/kg/day, was chosen as the maximum exposure concentration for the dose range-finding study.

Fifteen time-mated female rats were allocated to each exposure group. Viability, clinical observations, body weights, pup counts (litters were not standardized), and feed consumption were recorded to help determine the maximum exposure concentration that could be tolerated by the dams while not affecting the number of pups, so the MOG study could be populated with a sufficient number of offspring. Maternal plasma, amniotic fluid, and fetuses were collected from three dams per group on GD 18 for bioanalytical method development. On LD 4, maternal plasma was collected from dams with whole litter loss from the 1,875 ppm (two dams) and 3,750 ppm (one dam) groups. On PND 4, pup carcasses with heads were collected from the 0 (six pups), 1,875 (six pups), and 3,750 ppm (six pups) groups. On LD 28, maternal plasma was collected from three dams per group, and pup plasma was collected from three pups/sex/litter. Selected samples were analyzed for free (parent) and total (combined parent and conjugated forms) BPAF to determine the extent of maternal transfer and the internal dose to inform design of the MOG study. The corresponding data are reported elsewhere.⁴⁴

All other dams and pups were euthanized on LD 28 without further examination. Females that did not litter were euthanized approximately 3 days after expected littering, received a gross necropsy, and had their pregnancy status determined. If present, the numbers of implantation sites and ovarian corpora lutea were recorded. F₁ pups that were removed for health reasons or had died, and all females euthanized early in the 15,000 ppm group received a gross necropsy. Further details of animal maintenance and study design are given in Table 2.

Modified One-Generation Study with Prenatal, Reproductive Performance, and Subchronic Cohorts

Time-mated F₀ female rats, 35 per group, were received on GDs 1 or 2, randomized based on GD 3 body weight, and placed on a 5K96 Casein diet containing 0, 338, 1,125, or 3,750 ppm BPAF ad libitum on GD 6. The exposure concentration of 3,750 ppm was expected to result in minimal maternal toxicity and to ensure that the model system was appropriately challenged, increasing the likelihood of identifying any toxicological signal in the offspring. The F₁ and F₂ generations were exposed to BPAF via the dam during gestation and lactation, and directly via 5K96 feed at the same exposure concentration as their respective dams. Viability, clinical

observations, body weights, pup counts, and feed consumption were recorded. F₁ and F₂ litters were standardized to ten pups (5/sex/litter, when possible) and eight pups (4/sex/litter, when possible), respectively, on PND 4. At weaning on PND 28, F₁ offspring were randomly assigned to reproductive performance (1/sex/litter), prenatal development (1/sex/litter), subchronic (1/sex/litter from 10 litters), or biological sample collection (6/sex for sample collection on PND 28 to determine internal dose and up to 12 females for sample collection at vaginal opening [VO]) cohorts. In addition, 15 F₁ control males were reassigned as sentinels. F₁ animals that were not assigned to a cohort were considered extra animals and were euthanized on PND 28. The F₂ offspring were carried out to 91 days to allow for comparisons of similar parameters between the F₁ and F₂ generations. Information on feed composition and contaminants is provided in Appendix B. Additional details of animal maintenance and study design are given in Table 2 and Table 3.

Endocrine-sensitive and Pubertal Endpoints

AGD and corresponding body weight (for covariate analyses) were recorded for each F₁ and F₂ pup on PND 1. AGD was measured using a stereomicroscope with a calibrated ocular reticle. The distance between the midpoint of the anal opening to the caudal edge of the genital papilla was recorded and converted to millimeters (mm). F₁ and F₂ male pups were evaluated for retention of areolae/nipples on PND 13 and observed for testicular descent over 28 days, beginning on PND 14. Acquisition of balanopreputial separation (BPS), defined as complete retraction of the prepuce from the glans penis, was evaluated in all F₁ and F₂ males over 98 (F₁) or 75 (F₂) days, beginning on PND 35, and body weight was recorded upon BPS acquisition. External genitalia were examined for malformations and undescended testes (cryptorchidism). The acquisition of VO was evaluated in F₁ and F₂ females beginning on PND 23–24 until PND 39–41, and the corresponding body weight recorded upon VO acquisition.

Vaginal Cytology

Beginning on PND 82 (approximately 16 days before mating) and PND 75 for F₁ and F₂ females, respectively, vaginal lavages were collected from the F₁ females (in the prenatal, reproductive performance, and subchronic cohorts) and from F₂ females (in the reproductive performance cohort) for 16 consecutive days for evaluation of estrous cyclicity. Vaginal vaults were moistened with saline, if necessary, and samples of vaginal fluid and cells were spotted onto a slide and subsequently stained with toluidine blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (diestrus, proestrus, estrus, and metestrus).⁴⁵

F₁ Cohabitation and Assessment of Mating

Sexually mature F₁ animals in the prenatal (14–17 weeks; one male and one female per litter) and reproductive performance (13–18 weeks; one male and one female per litter) cohorts were randomly assigned a mating partner, avoiding sibling pairings, and paired in a 1:1 ratio for up to 15 days. Mating was confirmed by daily examination for the presence of a vaginal copulation plug or sperm in a vaginal lavage. The day of confirmed mating was considered GD 0. Females in the prenatal cohort that did not exhibit evidence of mating were euthanized with 100% carbon dioxide and necropsied at the end of cohabitation. Females in the reproductive performance cohort that did not exhibit evidence of mating, did not deliver a litter, or with whole litter loss were euthanized with 100% carbon dioxide and necropsied when F₂ pups reached PND 28. The uterus of apparently nonpregnant females was examined grossly and stained with ammonium

sulfide to identify potential implantation sites. The number of corpora lutea on the ovary were enumerated, and gross lesions were examined for histopathological changes.

Prenatal Cohort

On GD 21, pregnant F₁ females were euthanized with 100% carbon dioxide and F₂ fetuses were removed from the uterus, individually weighed (live fetuses only), and examined externally for alterations, including inspection of the oral cavity for cleft palate. Gross placental morphology was also evaluated. Live fetuses were subsequently euthanized by oral administration of sodium pentobarbital. F₁ females with no evidence of mating were euthanized with 100% carbon dioxide and necropsied and examined for gross lesions, which were retained and examined histologically. Fetal sex was confirmed by inspection of gonads in situ. All F₂ fetuses in each litter were examined for soft tissue alterations under a stereomicroscope.^{46; 47} The heads were removed from approximately half of the fetuses in each litter, fixed in Bouin's solution, and subsequently examined by freehand sectioning.⁴⁸ This technique precludes skeletal evaluations of the skull; therefore, remaining heads and all fetuses were eviscerated, fixed in ethanol, macerated in potassium hydroxide, stained with Alcian blue and Alizarin red, and examined for subsequent cartilage and osseous alterations.^{49; 50} External, visceral, and skeletal fetal findings were recorded as developmental variations or malformations. At the end of cohabitation, male sires were necropsied, selected organs were weighed, and gross lesions were collected for potential histological examination. In addition, due to clotting of some of the blood samples collected from males in the subchronic cohort at scheduled necropsy, blood samples from males in the prenatal cohort were collected at necropsy and used for micronucleus, hematology, and clinical chemistry evaluations.

Reproductive Performance Cohort

Fertility and fecundity were assessed in one male and one female representing each F₁ litter and all exposure groups. Pup viability was assessed daily during lactation. F₂ offspring were standardized to a litter size of eight pups (4/sex/litter, when possible) on PND 4. F₁ males were euthanized with 100% carbon dioxide at approximately 22 weeks of age after assessment of fertility, fecundity, and F₂ generation pup survival. The F₁ females and the F₂ offspring were euthanized with 100% carbon dioxide on PND 91–93, when the F₁ females were 23–25 weeks of age. F₂ offspring were carried out to PND 91–93 to determine whether any effects in the F₁ generation would be replicated. F₂ offspring were given a gross necropsy. F₁ sires were necropsied after completion of littering for the F₂ generation; selected organs were weighed, and gross lesions were collected for potential histological examination.

Immediately after euthanasia, the left testis and epididymis were removed, trimmed, and weighed. The cauda epididymis was then weighed, and samples were collected for determining cauda epididymal sperm motility, number, and density via automated sperm analyzer (Hamilton Thorne, Inc., Beverly, MA). The sampled left cauda epididymis and the intact corpus and caput were frozen at –80°C for subsequent determination of epididymal sperm concentration from the left cauda epididymis. The left testis was frozen at –80°C for subsequent determination of homogenization-resistant spermatid head counts for calculations of daily sperm production and efficiency of daily sperm production.⁵¹ The right testis and epididymis were examined histologically. Gross lesions took precedence over sperm parameter assessments (i.e., if the left testis was grossly abnormal, it and the left epididymis would be examined histologically, and the right testis and epididymis, if grossly normal, would be subjected to sperm assessments).

Subchronic Cohort

General toxicity was assessed in one male and one female representing 10 random litters (within an exposure concentration) and all exposure groups. F₁ males and females were euthanized and necropsied on PND 115–119 and PND 116–120, respectively. The animals were anesthetized with carbon dioxide and euthanized by exsanguination. Blood was collected by cardiac puncture. Approximately 500 µL of whole blood was collected into a tripotassium ethylenediaminetetraacetic acid (K₃ EDTA)-treated tube for hematology analyses. Up to 3 mL whole blood was collected into a serum separator tube for preparation of serum for clinical chemistry analyses. The samples for clinical pathology analyses were stored at 4°C until transferred to Antech[®] GLP (Morrisville, NC) on the same day as necropsy for the clinical pathology analyses. The parameters measured are listed in Table 3.

In addition, approximately 200 µL of whole blood was collected into a K₃ EDTA-treated tube for micronucleus determination. The micronucleus samples were stored at 4°C until transferred to the designated NTP laboratory (Integrated Laboratory Systems, LLC, Research Triangle Park, NC) on the same day as the necropsy.

Biological Sampling Cohort

On GD 18, maternal plasma, amniotic fluid, and fetuses were collected from three pregnant dams per group. On LD 4, maternal plasma was collected from three dams with litters from the 338 and 1,125 ppm groups. Pup plasma was collected on PND 4 from nine pups/sex/group (from at least three litters per group). On LD 28, maternal plasma was collected from three dams per group, and pup plasma was collected from three pups/sex/group. In addition, plasma was collected from six weanlings/sex/group on PND 28. Adult and PND 28 animals were euthanized with 100% carbon dioxide and PND 4 pups were decapitated or administered a solution containing sodium pentobarbital. Samples were analyzed for free (parent) and total (combined parent and conjugated forms) BPAF using a validated analytical method and published elsewhere.⁴⁴ In addition, at the time of VO, serum and brains were collected from up to 12 females per group from this cohort and frozen for potential future analyses. Ovaries (paired) were also collected and sent for standard histopathology evaluation (Experimental Pathology Laboratories, Inc., Research Triangle Park, NC).

Necropsy and Histopathology

Complete necropsies were performed on adult F₁ males and F₁ females in the subchronic and reproductive performance cohorts, unscheduled deaths, F₀ females, F₁ males and F₁ females in the prenatal cohort, F₁ females in the reproductive performance cohort that either had no evidence of mating or did not produce a litter, and F₂ offspring. All gross lesions were examined histologically. In addition, several protocol-required tissues were examined microscopically from the adult F₁ males and females in the prenatal, reproductive performance, and subchronic cohorts. Tissues from the F₂ animals in the reproductive performance cohort were collected and fixed but not evaluated. In the prenatal cohort, organ weights were recorded for the Cowper's glands (paired), epididymis (left and right), levator ani/bulbocavernosus (LABC) muscle, ovary (left and right), preputial glands (paired), dorsolateral and ventral prostate gland, seminal vesicles with coagulating glands, and testis (left and right). In the reproductive performance cohort, organ weights were recorded for the Cowper's glands (paired), epididymis (left and right), LABC muscle, ovary (left and right), preputial glands (paired), dorsolateral and ventral prostate gland, seminal vesicles with coagulating glands, testis (left and right), and brain

(F₂ only). In the subchronic cohort, organ weights were recorded for the adrenal glands (paired), epididymis (right and left), heart, kidney (right and left), liver, lungs, ovary (left and right), dorsolateral and ventral prostate gland, seminal vesicles with coagulating glands, testis (right and left), thymus, thyroid (fixed), and uterus with cervix and vagina.

The initial histological examination was performed on adult F₁ males and F₁ females in the subchronic and reproductive performance cohorts by an experienced, board-certified veterinary pathologist. The slides, individual animal data records, and pathology tables were subsequently evaluated by an independent quality assessment (QA) laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A QA pathologist evaluated selected slides from the various cohorts. The testes, epididymides, dorsolateral and ventral prostate, seminal vesicle, penis, ovaries, and uterus were reviewed from all animals in the F₁ reproductive performance and subchronic cohorts for which the tissue had been examined previously by the study laboratory pathologist. In addition, the LABC muscle, Cowper's glands, coagulating gland, and vagina were examined in the F₁ reproductive performance cohort, and the adrenal gland and kidneys were reviewed from all control and 3,750 ppm males and females in the F₁ subchronic cohort.

The QA report and the reviewed slides were submitted to the NTP pathologist, who reviewed and addressed any inconsistencies in the diagnoses made by the laboratory and QA pathologist. The QA pathologist, who served as the coordinator of the Pathology Working Group (PWG) presented representative histopathology slides containing examples of lesions related to test agent administration, examples of disagreements in diagnoses between the laboratory and QA pathologist, or lesions of general interest to the PWG for review. The PWG consisted of the NTP pathologist and other pathologists experienced in rodent toxicological pathology. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, QA pathologist, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman⁵² and Boorman et al.⁵³

Table 2. Experimental Design and Materials and Methods in the Dose Range-finding and Modified One-Generation Studies of Bisphenol AF (Prewaning)

Dose Range-finding Study	Modified One-Generation Study
Study Laboratory	
RTI International (Research Triangle Park, NC)	Same as dose range-finding study
Strain and Species	
Sprague Dawley (Hsd:Sprague Dawley® SD®) rats	Same as dose range-finding study
Animal Source	
Envigo (formerly Harlan Laboratories, Inc., Dublin, VA)	Same as dose range-finding study
Day of Arrival	
November 27, 2012 (GD 1 or GD 2)	May 7 or 9, 2013 (GD 1 or GD 2)
Average Age on Arrival	
11–12 weeks	Same as dose range-finding study
Weight Range at Randomization	
186.7–255.3 g on GD 3	187.5–260.7 g on GD 3
Date of First Exposure	
GD 6 (December 1, 2012)	F ₀ females: GD 6 (May 11–14, 2013) F ₁ rats (all cohorts): lifetime exposure F ₂ rats: lifetime exposure
Duration of Exposure	
GD 6 through LD 28	F ₀ females: GD 6 through LD 28 F ₁ rats (biosampling cohort): lifetime exposure through PND 28 (males and females) or until day of vaginal opening (females) F ₁ rats (subchronic cohort): lifetime exposure through PND 115–119 (males) or through PND 116–120 (females) F ₁ rats (prenatal cohort): lifetime exposure through PND 119–121 (males) or through PND 123–137 (females) F ₁ rats (reproductive performance cohort): lifetime exposure through PND 152–154 (males) or through PND 158–175 (females) F ₂ rats (reproductive performance cohort): in utero through PND 91–93
Date of Last Exposure	
LD 28 (January 16, 2013)	F ₀ females: LD 28 (June 24–28, 2013) F ₁ rats (biosampling cohort): PND 28 (June 24–27, 2013) or day of vaginal opening (June 24–July 7, 2013)

Bisphenol AF, NTP DART 08

Dose Range-finding Study	Modified One-Generation Study
	<p>F₁ rats (subchronic cohort): PND 115–119 (through September 23, 2013) (males) or PND 116–120 (through September 24, 2013) (females)</p> <p>F₁ rats (prenatal cohort): PND 119–121 (through September 25–27, 2013) (males) or PND 123–137 (through September 30–October 13, 2013) (females)</p> <p>F₁ rats (reproductive performance cohort): PND 152–154 (October 28–30, 2013) (males) or PND 158–175 (November 5–19, 2013) (females)</p> <p>F₂ rats (reproductive performance cohort): PND 91–93 (through January 7–21, 2014)</p>
<p>Necropsy Dates</p> <p>Gross necropsies were conducted on F₀ females euthanized early in the 15,000 ppm group, F₀ females that did not deliver a litter, and F₁ offspring euthanized moribund or found dead.</p>	<p>F₀ females: LD 28 (June 24–28, 2013)</p> <p>F₁ rats (biosampling cohort): June 24–27, 2013 (males and females) or June 24–July 7, 2013 (females on day of vaginal opening)</p> <p>F₁ rats (subchronic cohort): September 23–24, 2013</p> <p>F₁ rats (prenatal cohort): September 25–27, 2013 (males) or September 30–October 13, 2013 (females)</p> <p>F₁ rats (reproductive performance cohort): October 28–30, 2013 (males) or November 5–19, 2013 (females)</p> <p>F₂ rats (reproductive performance cohort): January 7–21, 2014</p>
<p>Average Age at Necropsy</p> <p>Not performed</p>	<p>F₀ females: ~19 weeks</p> <p>F₁ rats (biosampling cohort): 28 days (males and females), or 26–39 days (females on day of vaginal opening)</p> <p>F₁ rats (subchronic cohort): 115–119 days (males) or 116–120 days (females)</p> <p>F₁ rats (prenatal cohort): 119–121 days (males) or 123–137 days (females)</p> <p>F₁ rats (reproductive performance cohort): 152–154 days (males) or 158–175 days (females)</p> <p>F₂ rats (reproductive performance cohort): 91–93 days (males and females)</p>
<p>Size of F₀ Study Groups</p> <p>15 time-mated females</p>	<p>35 time-mated females</p>

Bisphenol AF, NTP DART 08

Dose Range-finding Study	Modified One-Generation Study
Method of Randomization and Identification	
Time-mated animals were individually identified by an implanted micro transponder (Bio Medic Data Systems, Seaford, DE) and assigned to exposure group by stratified randomization of GD 3 body weights using Provantis® (Instem, Stone, United Kingdom) electronic data collection system.	Same as dose range-finding study, except F ₁ and F ₂ pups were identified by paw tattoo, and postweaning F ₁ males and F ₁ females were identified by an implanted micro transponder.
Animals per Cage	
1 (with litter)	F ₀ females: 1 (with litter) F ₁ rats (biosampling cohort): ≤3 (males or females) until termination F ₁ rats (subchronic cohort): ≤3 (males or females) until termination F ₁ rats (prenatal cohort): ≤3 (males or females) until PND 98, then housed individually except during cohabitation F ₁ rats (reproductive performance cohort): ≤3 (males or females) until PND 98, then housed individually except during cohabitation or when housed with their litters F ₂ rats (reproductive performance cohort): ≤3 (males or females) until PND 91–93
Diet	
Irradiated certified Advanced Protocol Verified Casein Diet 1 IF 5K96 (PMI Nutrition International, Richmond, IN), available ad libitum	Same as dose range-finding study
Water	
Tap water (Durham, NC) via automatic watering system (Avidity Science, formerly Edstrom Industries, Inc., Waterford, WI), available ad libitum	Same as dose range-finding study
Cages	
Solid-bottom polycarbonate cages (Lab Products, Inc., Seaford, DE), rotated biweekly and changed at least once per week	Solid-bottom polycarbonate cages (Lab Products, Inc., Seaford, DE), rotated biweekly and changed at least once per week (individually housed animals) or twice per week (group-housed animals and females with litters)
Bedding	
Certified irradiated Sani-Chips® hardwood cage bedding (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly	Certified irradiated Sani-Chips® hardwood cage bedding (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly (individually housed animals) or twice per week (group-housed animals and females with litters)
Cage Filters	
Filter paper (Granville Milling Co., Creedmoor, NC), changed biweekly	Same as dose range-finding study

Bisphenol AF, NTP DART 08

Dose Range-finding Study	Modified One-Generation Study
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks during the study	Same as dose range-finding study
Animal Room Environment	
Temperature: 71.8°F ± 1.5°F Relative humidity: 52.6% ± 7.5% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72°F ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Exposure Concentrations	
0, 937.5, 1,875, 3,750, 7,500, or 15,000 ppm BPAF in feed, available ad libitum	0, 338, 1,125, or 3,750 ppm BPAF in feed, available ad libitum
Type and Frequency of Observation of F₀ and F₁ Dams	
Viability was assessed at least twice daily, and clinical observations were recorded at least once daily. Female body weights were recorded daily during gestation (GD 3–21) and during lactation on LDs 1, 4, 7, 11, 14, 18, 21, 25, and 28. Feed consumption was recorded at 3-day intervals from GD 3 through GD 21 and for LDs 1–4, 4–7, 7–11, 11–14, 14–18, 18–21, 21–25, and 25–28.	Viability was assessed at least twice daily, and clinical observations were recorded at least once daily. Female body weights were recorded daily during gestation (GD 3–21) and during lactation on LDs 1, 4, 7, 10, 13, 16, 19, 21, 25, and 28. Feed consumption was recorded at 3-day intervals from GD 3 through GD 21 and for LDs 1–4, 4–7, 7–10, 10–13, 13–16, 16–19, 19–21, 21–25, and 25–28.
Type and Frequency of Observation of F₁ and F₂ Pups	
Viability was assessed at least twice daily, and clinical observations were recorded at least once daily. The number of live and dead pups in each litter was counted daily. Individual pups were sexed and weighed on PNDs 1, 4, 7, 14, 21, 24, 25, and 28. Litters were not standardized on PND 4, and all offspring (unless euthanized and biological samples collected for subsequent analytical method development) were retained until PND 28 to assess litter size, sex distribution, pup body weights, and survival during lactation.	Viability was assessed at least twice daily, and clinical observations were recorded at least once daily. The number of live and dead pups in each litter was counted daily. Individual pups were sexed and weighed on PNDs 1, 4, 7, 10, 13, 16, 19, 21, 25, and 28. F ₁ and F ₂ litters were standardized to a litter size of 10 (5/sex/litter, when possible) and 8 pups (4/sex/litter, when possible), respectively, on PND 4. Endocrine F ₁ /F ₂ endpoints: AGD and corresponding pup weight on PND 1; areolae/nipple retention on PND 13; testicular descent beginning on PND 14
Primary Method of Euthanasia	
100% carbon dioxide (F ₀ females and PND 28 pups); decapitation (GD 18 fetuses; PND 4 pups)	100% carbon dioxide (adults and PND 28 pups); decapitation or administration of a solution containing sodium pentobarbital (PND 4 pups)
Necropsy and Postmortem Evaluation	
F ₀ dams and their pups were euthanized on LD 28 without necropsy. Females that did not litter were euthanized ~3 days after expected littering, received a gross necropsy, and had their pregnancy status determined. If present, the numbers of implantation sites and ovarian corpora lutea were recorded. F ₁ pups that were removed for health reasons or had died and all F ₀ females euthanized early in the 15,000 ppm group received a gross necropsy.	F ₀ dams were euthanized on LD 28, received a gross necropsy, and had their number of implantation sites and corpora lutea recorded. Females that did not litter were euthanized 3 days after expected littering, received a gross necropsy, and had their pregnancy status determined. If present, the number of implantation sites and corpora lutea was recorded. Histopathological analysis of gross lesions was performed, if collected.

Bisphenol AF, NTP DART 08

Dose Range-finding Study	Modified One-Generation Study
<p>Internal Dose Assessment</p> <p>On GD 18, maternal plasma, amniotic fluid, and fetuses were collected from three pregnant dams/group. On LD 4, maternal plasma was collected from dams with whole litter loss from the 1,875 ppm (two dams) and 3,750 ppm (one dam) groups. On PND 4, pup carcasses with heads were collected from the 0 (six pups), 1,875 (six pups), and 3,750 ppm (six pups) groups. On LD 28, maternal plasma was collected from three dams/group, and pup plasma was collected from 3 pups/sex/litter. Selected samples were analyzed for free (parent) and total (combined parent and conjugated forms) BPAF to determine the extent of maternal transfer and to inform the design of the MOG study. The corresponding data are not reported.</p>	<p>On GD 18, maternal plasma, amniotic fluid, and fetuses were collected from three pregnant dams per group. On LD 4, maternal plasma was collected from three dams with litters from the 338 and 1,125 ppm groups. Pup plasma was collected on PND 4 from 9 pups/sex/group (from at least 3 litters/group). On LD 28, maternal plasma was collected from 3 dams/group, and pup plasma was collected from 3 pups/sex/group. Samples were analyzed for free (parent) and total (combined parent and conjugated forms) BPAF using a validated analytical method and results were published elsewhere.⁴⁴</p>
<p>GD = gestation day; LD = lactation day; PND = postnatal day; BPAF = bisphenol AF; AGD = anogenital distance; MOG = modified one-generation.</p>	

Table 3. Experimental Design and Materials and Methods in the Modified One-Generation Study of Bisphenol AF (Postweaning)

Modified One-Generation Study
F₁ Postweaning Assessments
<p>All Cohorts: Viability was assessed at least twice daily, and clinical observations recorded at least once daily. F₁ male body weights and feed consumption were recorded once weekly. F₁ female body weights and feed consumption were recorded at least once weekly during the premating interval. Vaginal opening (and concomitant body weight) was evaluated beginning on PND 20, balanopreputal separation (and concomitant body weight) was evaluated beginning on PND 35.</p> <p>Prenatal and Reproductive Performance Cohorts: After collection of vaginal lavage samples for 16 days, F₁ nonsibling mating pairs (one male and one female per litter) from the same exposure group were cohabitated until evidence of mating or for ≤ 15 days. F₁ dams were observed for the same gestational endpoints as the F₀ dams.</p> <p>Prenatal Cohort: F₁ dams were evaluated at GD 21 necropsy, and F₂ fetuses were assessed for external, visceral, and skeletal variations and malformations.</p> <p>Reproductive Performance Cohort: F₁ dams and F₂ pups were evaluated for the same lactational endpoints as the F₀ dams and F₁ pups.</p>
F₁ Necropsy and Postmortem Evaluation
<p>Prenatal Cohort: F₁ dams were euthanized on GD 21. Necropsies were performed on all females. Terminal body weights and ovary (left and right) and gravid uterus weights were recorded. The number of corpora lutea on each ovary was recorded. The number and location of all fetuses and resorptions (early or late) and the total number of implantation sites were recorded. If there was no macroscopic evidence of pregnancy, the uterus was stained to visualize potential evidence of implantation sites. Live fetuses were counted, sexed, weighed, and examined for external morphological abnormalities, including examination of the oral cavity for cleft palate. Gross placental morphology was also evaluated. Live fetuses were euthanized and then examined for visceral morphological abnormalities by fresh dissection. The sex of each fetus was confirmed by internal examination. The heads from approximately one-half of the fetuses in each litter were fixed, sectioned, and examined. All fetuses were eviscerated, fixed, stained, and examined for skeletal developmental variations, malformations, or other morphological findings. After positive evidence of mating, male sires were weighed, euthanized, and necropsied, and the following organ weights recorded: Cowper's glands (paired), epididymis (left and right), LABC muscle, preputial glands (paired), dorsolateral and ventral prostate gland, seminal vesicles with coagulating glands, and testis (left and right). Histopathology of gross lesions was assessed.</p> <p>Reproductive Performance Cohort: F₁ dams were euthanized on LD 28, and sires were euthanized after assessment of fertility, fecundity, and F₂ generation pup survival. Terminal body weights and the following organ weights were recorded for the F₁ and F₂ males and females: Cowper's glands (paired), epididymis (left and right), LABC muscle, ovary (left and right), preputial glands (paired), dorsolateral and ventral prostate gland, seminal vesicles with coagulating glands, and testis (left and right). Histopathology was performed on the following organs for F₁ males and/or females: Cowper's glands, epididymis, LABC muscle, ovaries, pituitary gland, preputial glands, dorsolateral and ventral prostate gland, seminal vesicles with coagulating glands, testis, uterus (with cervix and vagina), and gross lesions. Cauda epididymal sperm motility, cauda epididymal sperm concentration, and testicular sperm head counts were also assessed for all F₁ and F₂ males. For the F₂ generation, organ weights were collected for the same organs as for the F₁ necropsy, with the brain also weighed. The same reproductive tissues were fixed, but histopathology was not evaluated for any tissues or gross lesions.</p> <p>Biological Sampling Cohort: Rats were randomly allocated for collection of biological samples. On PND 28, plasma was collected from 6 weanlings/sex/group. At the time of vaginal opening, serum, ovaries (paired), and brains were collected from up to 12 females/group and frozen for potential future analyses. Results of the plasma analyses have been reported previously.⁴⁴ Rats were subjected to a gross necropsy, and histopathology was performed on gross lesions.</p>

Modified One-Generation Study

Subchronic Cohort: F₁ males and females were euthanized on PND 115–119 and PND 116–120, respectively. Blood was collected by cardiac puncture and processed for hematology and clinical chemistry analyses. Additional blood samples were also collected for micronucleus determination. The following hematology parameters were analyzed: erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, leukocyte count, leukocyte differential, reticulocyte count, and platelet count. The following clinical chemistry parameters were analyzed: total protein, albumin, urea nitrogen, creatinine, alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, bile acids, glucose, creatine kinase, cholesterol, and triglycerides. The following organ weights were recorded: adrenal glands (paired), epididymis (right and left), heart, kidney (right and left), liver, lungs, ovary (left and right), dorsolateral and ventral prostate gland, seminal vesicles with coagulating glands, testis (right and left), thymus, thyroid (fixed), and uterus with cervix and vagina. In addition to gross lesions, histopathology was performed on the following organs: adrenal glands (paired), bone with marrow, brain, cervix, clitoral glands, epididymides (paired), esophagus, eyes, Harderian glands, heart and aorta, kidneys (paired), large intestine (cecum, colon, and rectum), liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovaries (paired), pancreas, parathyroid glands, pituitary gland, preputial glands, prostate gland, salivary glands, seminal vesicles with coagulating gland, small intestine (duodenum, jejunum, and ileum), spleen, stomach (forestomach and glandular), testes (paired), thymus, thyroid gland, trachea, urinary bladder, uterus, vagina, and Zymbal's glands.

PND = postnatal day; GD = gestation day; LABC = levator ani/bulbocavernosus; LD = lactation day.

Statistical Methods

Statistical methods were chosen based on distributional assumptions as well as on the need to incorporate within-litter correlation among animals. Unless specifically mentioned, all endpoints were tested for a trend across exposure groups, followed by pairwise tests for each exposure group against the control group. Significance of all trend and pairwise tests is reported at both 0.05 and 0.01 levels.

Analysis of Fetal Malformations and Variations

Incidences of malformations and variations in fetuses were summarized as number of litters affected and as number of fetuses affected. Trend and pairwise analyses of the fetal malformations and variations was conducted using a Cochran-Armitage test with a Rao-Scott adjustment, as described below.

The tendency of fetuses from the same litter to respond more similarly than fetuses from different litters has been referred to as the “litter effect”⁵⁴ and reflects littermates’ similarities in genetics and in utero experiences. Failure to account for correlation within litters leads to underestimates of variance in statistical tests, resulting in higher probabilities of Type I errors (“false positives”). Therefore, the Cochran-Armitage trend test for incidence data was modified to accommodate litter effects using the Rao-Scott approach.⁵⁵ The Rao-Scott approach accounts for litter effects by estimating the ratio of the variance in the presence of litter effects to the variance in the absence of litter effects. This ratio is then used to adjust the sample size downward to yield the estimated variance in the presence of litter effects. The Rao-Scott approach was implemented in the Cochran-Armitage test as recommended by Fung et al.,⁵⁶ formula \bar{T}_{RS2} .

Analysis of Incidences of Gross Pathology and Morphology Findings

For the F₀ dams, incidences of gross findings and histopathology were summarized as number of animals affected. Because some of these animals did not survive until the removal day for their

cohort, analysis of the histopathological findings was conducted using the Poly-3 test, as described below.

The Poly-k test⁵⁷⁻⁵⁹ was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage trend test to account for survival differences. Following Bailer and Portier,⁵⁷ a value of $k = 3$ was used in the analysis of site-specific lesions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams.⁶⁰ Poly-3 tests used the continuity correction described by Nam.⁶¹

For the F₁ and F₂ animals, incidences of gross findings and histopathology were summarized as number of litters affected and number of animals affected. To account for within-litter correlation, the Rao-Scott adjustment (as described earlier) was applied to the Cochran-Armitage test in the analysis of this data. For histopathology data in F₁ cohorts in which survival issues may apply, the Poly-3 correction was also applied.

All p values calculated for gross pathological and histopathological data are one-sided and include a continuity correction.

Analysis of Continuous Endpoints

Before statistical analysis, extreme values identified by the outlier test of Dixon and Massey⁶² for small samples ($n < 20$) and Tukey's outer fences method⁶³ for large samples ($n \geq 20$) were examined by NTP personnel, and implausible values were eliminated from the analysis.

In some instances, no considerations for litter effects were necessary in the analysis of the continuous data. This was the case for the F₀ generation and for the F₁ prenatal cohort for which there was only one animal per litter. In these instances, organ and body weight measurements, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett⁶⁴ and Williams.^{65; 66}

When litter effects were present, organ and body weight endpoints were analyzed using linear mixed models, with litters as a random effect. To adjust for multiple comparisons, a Dunnett-Hsu adjustment was used.⁶⁷ Pup and fetal weights were adjusted for litter size (see below). AGD was adjusted for the body weight of the pup taken on the day of AGD measurement. The adjusted AGDs were analyzed as normal variates with litter effects using a linear mixed model.

Feed consumption, litter sizes, pup survival, implantations, number of resorptions, uterine content endpoints, spermatid, and epididymal spermatozoal measurements typically have skewed distributions. When litter effects were not present, these endpoints were analyzed using the nonparametric multiple comparison methods of Shirley⁶⁸ (as modified by Williams⁶⁹ and Dunn⁷⁰). For these endpoints, the Jonckheere test⁷¹ was used to assess the significance of the exposure concentration-related trends and to determine, at the 0.01 level of significance, whether a trend-sensitive test (the Williams or Shirley test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic exposure concentration-related trend (the Dunnett or Dunn test).

When litter effects were present for non-normally distributed continuous endpoints, the trend across exposure groups was analyzed by a permutation test based on the Jonckheere trend test

implemented by randomly permuting whole litters across exposure groups and bootstrapping within the litters (see, for example, Davison and Hinckley⁷²). Pairwise comparisons were made using a modified Wilcoxon test that incorporated litter effects.⁷³ The Hommel procedure was used to adjust for multiple comparisons.⁷⁴

Analysis of Feed Consumption Data

Feed consumption was measured at 3-day intervals for F₀ and F₁ dams during gestation and lactation and at least weekly thereafter. In some cases, consumption is reported over intervals that span multiple measurements (e.g., GD 6–21 and LD 1–14). These long-interval values are calculated at the animal or cage level using a weighted average of available constituent subinterval measurements, which are weighted by the underlying subinterval lengths. When spillage is noted or an outlier value is removed from the analysis, the subinterval value for the animal is not reported, and the long interval is calculated excluding that subinterval. As a result, there may be instances in which more animals are reported for a long interval (e.g., GD 6–21) than are reported for the constituent subintervals (e.g., GD 6–9, GD 9–12).

Analysis of Gestational and Fertility Indices

When litter effects were not present, Cochran-Armitage trend tests were used to test the significance of trends in gestational and fertility indices across exposure groups. Fisher's exact test was used to conduct pairwise comparisons of each exposed group with the control group. P values for these analyses are two-sided.

Body Weight Adjustments

Because body weights typically decrease with increasing litter size, adjusting body weight for litter size in the analysis of fetal and pup weights can provide additional precision to detect test article effects.⁷⁵ Body weight adjustments are appropriate when the litter effect, as evidenced by decreasing weights with increasing litter size, is relatively constant across exposure concentrations. Adjusted fetal weights were calculated by fitting a linear model to litter mean fetal weights as a function of litter size and exposure concentration, and the estimated coefficient of litter size was then used to adjust each litter mean fetal weight based on the difference between its litter size and the mean litter size. Preweaning pup body weights were adjusted for live litter size by fitting a linear model to body weights as a function of exposure concentration and litter size, with the coefficient of litter size retained for adjustment as above. Prestandardization PND 4 body weights were adjusted for PND 1 litter size, and body weights measured between PND 4 poststandardization and PND 21 were adjusted for PND 4 poststandardization litter size. After adjustment, mean body weights were analyzed with a linear mixed model with a random litter effect.

Analysis of Time-to-Event Data

Time-to-event endpoints, such as day of attainment of testicular descent, BPS, and VO, have a number of features that require careful model selection: non-normality of distributions, litter-based correlation, and censored values, meaning attainment was not observed before the end of the observation period. Further, growth retardation, reflected in the weaning weight, is an important covariate in the case of BPS and VO given the relationship between normal day of expected attainment and body weight.

When attainment times were approximately normally distributed and attainment was observed for all animals, two approaches for modeling discrete developmental endpoints were taken. First, a mixed model was fit to attainment day as a function of exposure concentration with a random litter effect. For BPS and VO, a second mixed model was fit to attainment day as a function of exposure concentration and weaning weight with a random litter effect. Dunnett-Hsu adjustments were used to account for multiple comparisons.

If censored observations were observed, survival analysis methods were used. In this case, a Cox proportional hazards model was fit with exposure concentration and weaning weight as covariates, a random effect for litter, and a Hommel adjustment for multiple comparisons.

To calculate mean attainment values adjusted for weaning weight, a linear model was fit to attainment day as a function of exposure concentration and weaning weight. The estimated coefficient of weaning weight was then used to adjust each attainment day based on the difference between the measured weaning weight and the mean weaning weight.

Cumulative response percentage, obtained using the methods of Kaplan-Meier, was plotted against time to attainment for unadjusted attainment times as well as attainment times adjusted for weaning weight. For litter-based plots, the litter median was used as time to attainment if >50% of the pups for that litter attained. Otherwise, litters with $\leq 50\%$ of the pups attaining had time to attainment set to the final day of observation. These litters are included in the denominator of Kaplan-Meier calculations but not the numerator.

Analysis of Vaginal Cytology Data

Vaginal cytology data consist of daily observations of estrous cycle stages over a 16-day period. Differences from the control group for cycle length and number of cycles were analyzed using a Datta-Satten modified Wilcoxon test with a Hommel adjustment for multiple comparisons.

To identify disruptions in estrous cyclicity, a continuous-time Markov chain model (multi-state model) was fit using a maximum likelihood approach,⁷⁶ producing estimates of stage lengths for each exposure group. Confidence intervals for these estimates were obtained based on bootstrap sampling of the individual animal cycle sequences. Stage lengths that were significantly different from the control group were identified using permutation testing with a Hommel adjustment.

Historical Control Data

The concurrent control group is the most valid comparison to the exposed groups and is the only control group analyzed statistically in NTP developmental and reproductive toxicity studies. However, historical control data are often helpful in interpreting potential exposure concentration-related effects, particularly for uncommon fetal findings that occur at a very low incidence. For meaningful comparisons, the conditions for studies in the historical control database must be generally similar. Factors that might affect the background incidences of fetal findings at a variety of sites are diet, strain/stock, route of exposure, study type, and/or laboratory that conducted the study. The NTP historical control database for fetal findings contains all fetal evaluations from teratology studies and/or modified one-generation studies for each laboratory. In general, the historical control database for a given study includes studies using the same route of administration and study design. However, historical control data for rats in this NTP Developmental and Reproductive Toxicity Technical Report contain data from feed and gavage

(all routes) studies conducted at RTI International. The concurrent controls are included in the historical control data set. NTP historical controls are available online at <https://ntp.niehs.nih.gov/data/controls/index.html>.

Quality Assurance Methods

This study was conducted in compliance with the Food and Drug Administration's Good Laboratory Practice for Nonclinical Laboratory Studies (Title 21, Part 58 of the Code of Federal Regulations).⁷⁷ In addition, this study was audited retrospectively by an independent QA assessment contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Developmental and Reproductive Toxicity Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this report.

Genetic Toxicology

The genetic toxicology of BPAF was assessed by testing whether the chemical induces mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases the frequency of micronucleated erythrocytes in rat peripheral blood. The protocol for these studies and the results are given in Appendix D.

The genetic toxicity studies have evolved from an earlier effort by NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the relationship between the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). The short-term tests were developed originally to clarify proposed mechanisms of chemical-induced DNA damage, given the relationship between electrophilicity and mutagenicity,⁷⁸ and the somatic mutation theory of cancer.^{79; 80} Not all cancers, however, arise through genotoxic mechanisms.

Bacterial Mutagenicity

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites.⁸¹ A positive response in the *Salmonella* test was shown to be the most predictive in vitro indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens).^{82; 83} Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. Other tests, however, can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

Peripheral Blood Micronucleus Test

Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division.^{84;}⁸⁵ Acute in vivo bone marrow chromosome aberration and micronucleus tests appear to be less predictive of carcinogenicity than the *Salmonella* test.^{86; 87} However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity;

a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies.⁸⁸ Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of in vivo genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

Results

Data Availability

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: <https://doi.org/10.22427/NTP-DATA-DART-08>.⁸⁹

Dose Range-finding Study

Maternal Findings

Viability and Clinical Observations

All F₀ rats in the 15,000 ppm group were euthanized on gestation day (GD) 10 or 11 due to body weight loss (Appendix E). Clinical observations of toxicity were limited to the 7,500 and 15,000 ppm groups and included eye and/or nasal discharge from GD 7 to GD 11 (Appendix E).

Body Weights and Feed Consumption

All F₀ exposed groups exhibited significant decreases of mean body weights starting within the first few days of exposure to bisphenol AF (BPAF), and the decreases were maintained throughout most of gestation. Mean body weights of dams in the 3,750 and 7,500 ppm groups were significantly decreased on GD 21 by 12% and 24%, respectively, compared to the control group, whereas mean body weights of the 937.5 and 1,875 ppm groups were approximately 5% lower (Table 4; Figure 5). The mean body weight gains of dams in the 937.5, 1,875, 3,750, and 7,500 ppm groups over the gestation period (GD 6–21) were lower by 23%, 19%, 31%, and 82%, respectively, compared to the control group, with significant decreases observed in the 3,750 and 7,500 ppm groups (Table 4; Figure 5). These decreases were attributed to body weight losses over the first half of gestation for the ≤3,750 ppm groups and a continual loss over the entire gestation period for the 7,500 ppm group (Table 4).

Mean body weights during lactation were significantly decreased in all exposed groups, relative to the control group, at the beginning of the lactation period. By lactation day (LD) 21, mean body weights of the 937.5, 1,875, and 3,750 ppm groups had recovered to near control group values. Mean body weights during lactation of the 7,500 ppm group were significantly decreased by 12% relative to that of the control group on LD 21 (Table 4; Figure 5).

Feed consumption during gestation by the exposed groups was highly variable compared to that of the control group, and it is likely that feed wastage (dams digging and spilling feed that could not be measured) contributed to the fluctuating levels across feed measurement intervals and decreased confidence in the accuracy of the respective BPAF feed consumption data (Table 5). The high level of feed wastage across the groups exposed to ≥1,875 ppm BPAF suggests that the feed was not very palatable, and a period of adjustment was required before the animals would consume the feed. BPAF intakes in the 937.5, 1,875, 3,750, and 7,500 ppm groups, based on measured feed consumption and dietary concentrations for GD 6–21, were approximately 56, 144, 368, and 618 mg BPAF/kg body weight/day (mg/kg/day), respectively (Table 5).

Bisphenol AF, NTP DART 08

Feed wastage also was observed during the lactation period but to a lesser extent (Table 5). BPAF intake in the 937.5, 1,875, 3,750, and 7,500 ppm groups, based on measured feed consumption and dietary concentrations for LD 1–14, was approximately 133, 348, 778, and 1,204 mg/kg/day, respectively (Table 5).

Table 4. Summary of Mean Body Weights and Body Weight Gains of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation and Lactation (Dose Range-finding Study)

Parameter ^{a,b}	0 ppm	937.5 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm
Gestation Body Weight						
Gestation Day						
6	238.9 ± 3.3 (12)	233 ± 5.0 (7)	237.0 ± 4.1 (13)	234.0 ± 4.2 (10)	240.9 ± 4.0 (10)	234.5 ± 3.4 (12)
9	251.2 ± 3.5** (12)	238.7 ± 4.7 (7)	239.2 ± 4.1* (13)	234.2 ± 3.7** (10)	230.1 ± 4.6** (10)	216.1 ± 3.6** (12)
12	266.2 ± 4.1** (12)	244.6 ± 5.5** (7)	246.3 ± 3.9** (13)	235.7 ± 2.8** (10)	223.8 ± 5.1** (10)	– ^c
15	286.2 ± 5.1** (12)	257.7 ± 6.1** (7)	263.5 ± 3.9** (13)	254.9 ± 4.0** (10)	243.1 ± 4.6** (10)	–
18	321.3 ± 8.3** (12)	293.4 ± 8.6* (7)	297.5 ± 4.7** (13)	287.5 ± 4.9** (10)	260.7 ± 5.3** (10)	–
21 ^d	347.0 ± 15.6** (9)	326.7 ± 8.5 (4)	329.3 ± 8.4 (10)	306.3 ± 4.0* (7)	263.0 ± 10.4** (7)	–
Gestation Weight Change						
Gestation Day Interval						
6–21 ^d	110.9 ± 13.7** (9)	84.9 ± 4.0 (4)	90.1 ± 5.7 (10)	76.6 ± 6.8* (7)	19.6 ± 12.8** (7)	–
6–9	12.3 ± 1.0** (12)	5.7 ± 1.4** (7)	2.2 ± 0.7** (13)	0.3 ± 1.4** (10)	–10.8 ± 2.3** (10)	–18.4 ± 2.1** (12)
9–12	15.0 ± 1.3** (12)	5.9 ± 1.8** (7)	7.1 ± 1.4** (13)	1.5 ± 1.8** (10)	–6.4 ± 2.5** (10)	–
12–15	19.9 ± 1.6 (12)	13.1 ± 2.1 (7)	17.2 ± 1.3 (13)	19.2 ± 1.7 (10)	19.3 ± 3.1 (10)	–
15–18	35.1 ± 4.2** (12)	35.6 ± 2.8 (7)	34 ± 1.5 (13)	32.6 ± 1.4 (10)	17.7 ± 5.3** (10)	–
18–21	31.4 ± 5.7** (9)	19.3 ± 2.1 (4)	29.6 ± 3.2 (10)	22.5 ± 3.2 (7)	3.1 ± 9.6** (7)	–
Lactation Body Weight						
Lactation Day						
1	258.3 ± 8.1** (7) ^e	219.0 ± 2.3** (4)	224.0 ± 5.5** (10)	217.0 ± 7.9** (7)	208.6 ± 7.2** (6) ^e	–
4	279.3 ± 5.5** (7)	240.7 ± 5.5** (4)	240.2 ± 6.2** (10)	233.7 ± 5.8** (7)	217.8 ± 8.7** (6)	–

Bisphenol AF, NTP DART 08

Parameter ^{a,b}	0 ppm	937.5 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm
7	293.0 ± 5.6** (7)	247.1 ± 9.5** (4)	267.1 ± 5.6** (8) ^f	252.1 ± 6.5** (6) ^f	229.4 ± 8.7** (6)	–
14	301.1 ± 4.7** (7)	275.4 ± 5.9* (4)	280.9 ± 4.4** (8)	272.8 ± 3.9** (6)	241.0 ± 6.5** (6)	–
21	283.7 ± 6.2 (7)	270.5 ± 7.4 (4)	293.6 ± 5.9 (8)	284.5 ± 5.2 (6)	250.6 ± 7.6** (6)	–
Lactation Weight Change						
Lactation Day Interval						
1–28	9.3 ± 7.4* (7)	50.4 ± 3.4** (4)	42.8 ± 5.2** (8) ^f	56.6 ± 11.4** (6) ^f	41.8 ± 5.5* (5) ^{e,f}	–
1–4	21.0 ± 3.4* (7)	21.7 ± 5.0 (4)	16.2 ± 2.4 (10)	16.8 ± 2.8 (7)	9.2 ± 4.3 (6)	–
4–7	13.7 ± 2.2 (7)	6.4 ± 8.1 (4)	20.4 ± 3.2 (8)	18.0 ± 5.1 (6)	11.6 ± 4.2 (6)	–
7–11	7.2 ± 2.9 (7)	19.4 ± 11.3 (4)	12.3 ± 1.3 (8)	18.0 ± 2.5 (6)	7.0 ± 2.5 (6)	–
11–14	0.9 ± 1.8 (7)	8.9 ± 3.3 (4)	1.5 ± 3.4 (8)	2.7 ± 2.9 (6)	4.6 ± 3.1 (6)	–
14–18	–10.3 ± 4.5 (7)	8.7 ± 2.5* (4)	8.3 ± 4.9** (8)	4.1 ± 2.0 (6)	9.0 ± 3.4** (6)	–
18–21	–7.0 ± 4.8 (7)	–13.6 ± 7.7 (4)	4.5 ± 3.4 (8)	7.7 ± 2.5 (6)	0.6 ± 4.6 (6)	–
21–25	–6.5 ± 2.4 (7)	3.5 ± 2.2 (4)	–10.9 ± 3.1 (8)	–5.0 ± 3.8 (6)	–3.1 ± 5.9 (5)	–
25–28	–9.6 ± 4.3 (7)	–4.6 ± 2.1 (4)	–12.0 ± 4.2 (8)	–6.7 ± 3.1 (6)	4.1 ± 1.8 (5)	–

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData are presented as mean ± standard error (n); body weight data are presented in grams.

^bStatistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^cThe 15,000 ppm group was removed on gestation day (GD) 10 or 11 due to excessive body weight loss.

^dDecreased number of dams at GD 21 reflects animals removed at GD 18 for biological sample collection.

^eDams not delivering with evidence of pregnancy were removed on GD 24 (two dams in the vehicle control group and one dam in the 7,500 ppm group).

^fDams with whole litter loss were removed on lactation day (LD) 4 for biological sample collection (two dams in the 1,875 ppm group, one dam in the 3,750 ppm group) and LD 21 (one dam in the 7,500 ppm group).

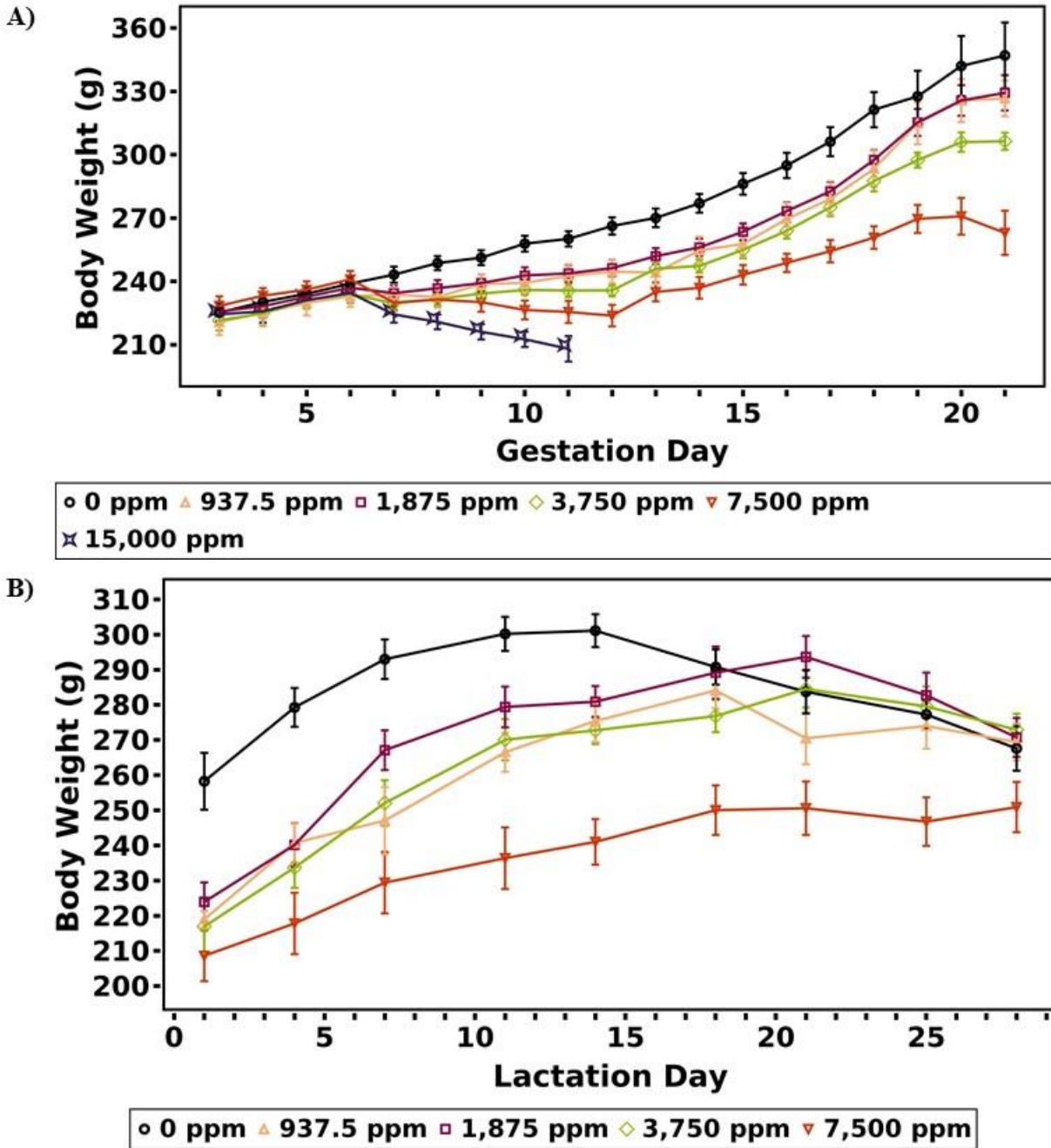


Figure 5. Growth Curves for F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation and Lactation (Dose Range-finding Study)

Growth curves are shown for (A) gestation and (B) lactation. Information for statistical significance in maternal weights is provided in Table 4.

Table 5. Summary of Feed and Test Article Consumption of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation and Lactation (Dose Range-finding Study)

Parameter ^{a,b,c}	0 ppm	937.5 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm
Feed Consumption (g/animal/day)^d						
Gestation Day Interval						
6–21	20.0 ± 0.6* (9)	16.2 ± 0.7 (4)	20.8 ± 1.2 (10)	25.0 ± 1.4* (7)	20.3 ± 2.3 (7)	– ^e
6–9	18.2 ± 0.4** (12)	14.5 ± 1.1 (7)	29.1 ± 3.9 (11)	38.6 ± 3.7** (9)	39.8 ± 2.2** (7)	32.2 ± 4.5** (8)
9–12	19.1 ± 0.5** (12)	14.9 ± 0.9** (7)	14.5 ± 0.6** (13)	13.5 ± 0.7** (9)	10.7 ± 1.4** (10)	–
12–15	19.7 ± 0.5 (12)	15.4 ± 0.6* (5)	20.1 ± 3.2 (8)	34.4 ± 1.0 (2)	25.6 ± 8.0 (2)	–
15–18	22.4 ± 0.8** (12)	19.8 ± 0.6** (7)	18.4 ± 0.6** (13)	19.8 ± 1.0** (10)	14.3 ± 1.2** (10)	–
18–21 ^f	20.6 ± 1.0 (9)	16.0 ± 1.3 (4)	18.7 ± 1.5 (9)	19.3 ± 0.9 (5)	30.3 ± 2.2 (4)	–
Lactation Day Interval						
1–14	49.7 ± 1.4 (7) ^g	35.5 ± 7.5 (4)	48.4 ± 3.6 (8) ^h	51.8 ± 4.1 (6) ^h	35.6 ± 5.1 (6) ^g	–
1–4	34.0 ± 1.0 (7)	22.5 ± 7.8 (2)	26.8 ± 3.0 (3)	43.6 ± 1.6 (2)	34.0 ± 4.3 (5)	–
4–7	46.4 ± 1.3* (7)	30.6 ± 3.4 (4)	41.3 ± 5.1 (8)	42.5 ± 6.8 (5)	24.8 ± 1.6** (4)	–
7–11	53.7 ± 1.5 (7)	56.5 ± 0.1 (2)	54.2 ± 4.1 (7)	60.0 ± 3.8 (6)	39.5 ± 6.0 (5)	–
11–14	63.1 ± 2.8** (7)	39.7 ± 11.7 (4)	55.3 ± 1.8 (8)	49.9 ± 5.4 (6)	28.1 ± 4.3** (6)	–
Chemical Intake (mg/kg/day)^{ij}						
GD 6–21	0.0 ± 0.0 (9)	56.2 ± 3.2 (4)	144.3 ± 7.0 (10)	367.8 ± 19.6 (7)	617.5 ± 69.1 (7)	–
LD 1–14	0.0 ± 0.0 (7)	133.1 ± 27.6 (4)	347.7 ± 23.6 (8)	777.5 ± 54.8 (6)	1,203.8 ± 206.7 (6)	–

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

GD = gestation day; LD = lactation day.

^aData are presented as mean ± standard error (n), where n = the number of dams. Feed consumption is not reported for nonpregnant animals during the gestation or lactation phase.

^bChanges in n are the result of excluded feed consumption values due to excessive spillage. Additional animal feed consumption values removed as outliers include: GD 9–12 (one value in the 3,750 ppm group), GD 18–21 (one value in the 3,750 ppm group), LD 4–7 (one value in the 7,500 ppm group), and LD 7–11 (one value in the 937.5 ppm group).

^cFor each dam, calculation of consumption values for the GD 6–21 and LD 1–14 intervals was performed using all valid data for the animal, even if data were unavailable for some of the subintervals.

^dStatistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

^eThe 15,000 ppm group was removed on GD 10 or 11 due to excessive body weight loss.

^fThree dams were removed in each group on GD 18 for biological sample collection.

^gDams not delivering with evidence of pregnancy were removed on GD 24 (two dams in the vehicle control group and one dam in 7,500 ppm group).

^hDams with whole litter loss were removed on LD 4 for biological sample collection (two dams in the 1,875 ppm group, one dam in the 3,750 ppm group).

ⁱChemical intake calculated as: $([\text{exposure concentration} \times \text{feed consumption}] / [\text{average body weight of day range}])$.

^jNo statistical analysis performed on the chemical intake data.

Maternal Reproductive Performance

Twenty-six out of 90 time-mated F₀ females were not pregnant: three in the control group, eight in the 937.5 ppm group (leaving four litters with uneven litter sizes for this group, which might have influenced the litter results), two in the 1,875 ppm group, five each in the 3,750 and 7,500 ppm groups, and three in the 15,000 ppm group (Table 6). There was no effect of BPAF exposure on the proportion of dams that produced viable litters, on gestation length, or on sex ratio. There was a negative trend in the BPAF-exposed groups for initial mean pups per litter (Appendix E), and LD 1 pup mean body weights were significantly decreased in the 937.5, 3,750, and 7,500 ppm groups compared to those of the control group.

Table 6. Summary of the Reproductive Performance of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation (Dose Range-finding Study)

Parameter ^a	0 ppm	937.5 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm
Time-mated Females (GD 6)	15	15	15	15	15	15
Females Pregnant (%)	12 (80.0)	7 (46.7)	13 (86.7)	10 (66.7)	10 (66.7)	12 (80.0)
Females Not Pregnant (%)	3 (20.0)	8 (53.3)	2 (13.3)	5 (33.3)	5 (33.3)	3 (20.0)
Dams Removed on GD 18 ^b	3	3	3	3	3	— ^c
Dams Not Delivering with Evidence of Pregnancy (%)	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	12 (100.0)
Dams with Litters on LD 0 (%) ^d	7 (77.8)	4 (100.0)	10 (100.0)	7 (100.0)	6 (85.7)	0 (0.0)
Gestation Length (days) ^{e,f,g}	22 ± 0.0 (7)	22.3 ± 0.3 (4)	22.3 ± 0.2 (10)	22.1 ± 0.1 (7)	22 ± 0.4 (6)	—
Live Litter Size on LD 0 ^{e,g}	13.3 ± 0.4 (7)	10.3 ± 1.7 (4)	12.5 ± 0.8 (8) ^h	11.3 ± 0.9 (7)	9.8 ± 1.5 (6)	—
LD 1 Pup Weight ^{g,i,j}	6.84 ± 0.19** 93 (7)	5.31 ± 0.40** 33 (4)	6.23 ± 0.15 98 (8)	5.77 ± 0.21* 70 (6) ^h	4.61 ± 0.38** 40 (6)	—
Percent Live Male Pups per Litter ^{e,g}	56.13 ± 5.86 (7)	60.26 ± 4.85 (4)	49.36 ± 3.19 (8)	51.70 ± 6.05 (7)	56.16 ± 10.27 (6)	—

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

GD = gestation day; LD = lactation day.

^aAnimals removed from the study between mating and littering were excluded from calculations of % littered females.

^bDams removed on GD 18 for biological sample collection.

^cThe 15,000 ppm group was removed on GD 10 or 11 due to excessive body weight loss.

^dPercentage is the number of littered females/pregnant females. Statistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.

^eStatistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

^fGestation length calculated for time-mated females that delivered a litter.

^gData are displayed as mean ± standard error (n).

^hChanges in n are the result of removing litters with no surviving pups by: LD 0 (two litters in the 1,875 ppm group) and LD 1 (one litter in the 3,750 ppm group).

ⁱn = the number of pups examined (number of litters).

^jStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

F₁ Offspring Findings

Pup Viability and Body Weights

BPAF exposure was associated with lower mean number of live pups per litter for all BPAF-exposed groups relative to the control group (Table 7; Appendix E). The number of dead pups during the lactation period (postnatal day [PND] 1–28) was 4, 20, 15, 26, and 34 for the control, 937.5, 1,875, 3,750, and 7,500 ppm groups, respectively (Table 7). Male pup mean body weight gains over the PND 1–28 interval in all exposed groups were significantly decreased (13%–58%) relative to the control pups, whereas female pup mean body weight gains were only significantly decreased compared to the control pups for the 3,750 and 7,500 ppm groups (14%–66%) (Table 8; Figure 6, Figure 7). Adverse F₁ pup clinical observations in all BPAF-exposed groups were consistent with the effects of BPAF exposure on pup survival (Appendix E). Findings included observations of pups found dead, cannibalized, missing, no milk band, dehydrated, bruised, stained fur, pale, cold to touch, or emaciated. There were no notable gross findings in the limited number of F₁ offspring that received a necropsy. Necropsy findings for pups found dead on or after PND 1 were limited to the absence of milk/feed in the stomach and one animal in the 1,875 ppm group with a distended ureter (Appendix E).

Table 7. Summary of F₁ Litter Size and Pup Survival Following Perinatal Exposure to Bisphenol AF (Dose Range-finding Study)

Postnatal Day	0 ppm	937.5 ppm	1,875 ppm	3,750 ppm	7,500 ppm
No. of Live Pups (Litters)^a					
0	93 (7)	41 (4)	100 (10)	79 (7)	59 (6)
Total Litter Size^{b,c}					
0	13.6 ± 0.4* (7)	12.5 ± 0.5 (4)	11.7 ± 1.0 (10)	11.7 ± 0.8 (7)	10.5 ± 1.4 (6)
Live Litter Size^{b,c}					
0	13.3 ± 0.4 (7)	10.3 ± 1.7 (4)	12.5 ± 0.8 (8) ^d	11.3 ± 0.9 (7)	9.8 ± 1.5 (6)
1	13.3 ± 0.4 (7)	8.3 ± 2.9 (4)	12.3 ± 1.0 (8)	11.7 ± 1.0 (6) ^d	6.7 ± 2.3 (6)
4	13.1 ± 0.5* (7)	7.5 ± 3.0 (4)	11.6 ± 1.0 (8)	11.5 ± 0.9 (6)	6.2 ± 2.2* (6)
7	12.0 ± 0.4* (7)	6.8 ± 2.8 (4)	10.3 ± 0.7 (8)	9.5 ± 1.1 (6)	6.2 ± 2.2* (6)
14	11.9 ± 0.5** (7)	5.3 ± 2.7* (4)	9.9 ± 0.6* (8)	7.8 ± 1.1** (6)	4.7 ± 1.6** (6)
21	11.9 ± 0.5** (7)	5.3 ± 2.7* (4)	9.9 ± 0.6* (8)	7.8 ± 1.1** (6)	5.0 ± 1.6** (5) ^d
28	11.9 ± 0.5** (7)	5.3 ± 2.7* (4)	9.9 ± 0.6* (8)	7.8 ± 1.1** (6)	5.0 ± 1.6** (5)
No. of Dead Pups (Litters)^a					
0	2 (1)	9 (2)	17 (5)	3 (3)	4 (1)
1–4	1 (1)	11 (3)	7 (3)	10 (2)	22 (6)
5–28	3 (2)	9 (2)	8 (5)	16 (5)	12 (3)
1–28	4 (2)	20 (4)	15 (7)	26 (6)	34 (6)
Dead/Litter^{b,c}					
0	0.29 ± 0.29 (7)	2.25 ± 1.31 (4)	1.70 ± 0.83 (10)	0.43 ± 0.20 (7)	0.67 ± 0.67 (6)
1–4	0.14 ± 0.14* (7)	2.75 ± 1.31 (4)	0.88 ± 0.61 (8) ^d	1.43 ± 1.27 (7)	3.67 ± 1.31** (6)

Bisphenol AF, NTP DART 08

Postnatal Day	0 ppm	937.5 ppm	1,875 ppm	3,750 ppm	7,500 ppm
5–28	0.43 ± 0.30 (7)	2.25 ± 1.44 (4)	1.00 ± 0.38 (8)	2.67 ± 0.95 (6) ^d	2.00 ± 1.18 (6)
1–28	0.57 ± 0.43** (7)	5.00 ± 1.47* (4)	1.88 ± 0.67* (8)	3.71 ± 1.25* (7)	5.67 ± 1.23** (6)
Survival Ratio^{b,c}					
0	0.98 ± 0.02 (7)	0.81 ± 0.11 (4)	0.77 ± 0.13 (10)	0.96 ± 0.02 (7)	0.94 ± 0.06 (6)
1–4	0.99 ± 0.01* (7)	0.63 ± 0.20 (4)	0.93 ± 0.04 (8) ^d	0.85 ± 0.14 (7)	0.56 ± 0.15** (6)
5–28	0.96 ± 0.03 (7)	0.70 ± 0.18 (4)	0.92 ± 0.03 (8)	0.74 ± 0.10 (6) ^d	0.68 ± 0.17 (6)
1–28	0.95 ± 0.04** (7)	0.43 ± 0.19* (4)	0.86 ± 0.05* (8)	0.65 ± 0.13* (7)	0.4 ± 0.11** (6)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

The 15,000 ppm group was removed on gestation day 10 or 11 due to excessive body weight loss.

^an = the number of pups (number of litters). For No. of Dead Pups, n is the number of litters contributing dead pups.

^bData are displayed as mean ± standard error of the litter means (n), where n = number of litters.

^cF₁ litter size and survival endpoints were analyzed using the Jonckheere (trend) and Shirley or Dunn (pairwise) tests. All calculations are based on the last litter observation of the day.

^dChanges in n for live litter size calculations are the result of removing litters with no surviving pups by: postnatal day (PND) 0 (two litters in the 1,875 ppm group), PND 1 (one litter in the 3,750 ppm group), and PND 17 (one litter in the 7,500 ppm group).

Table 8. Summary of F₁ Male and Female Pup Mean Body Weights and Body Weight Gains Following Perinatal Exposure to Bisphenol AF (Dose Range-finding Study)^{a,b}

Postnatal Day ^c	0 ppm	937.5 ppm	1,875 ppm	3,750 ppm	7,500 ppm
Male					
1	7.06 ± 0.21** 52 (7) ^d	5.62 ± 0.37** 18 (3)	6.32 ± 0.16* 49 (8)	5.81 ± 0.20** 39 (6)	4.82 ± 0.24** 22 (5)
4	9.87 ± 0.37** 51 (7)	6.22 ± 1.37** 17 (3)	8.33 ± 0.35 47 (8)	7.79 ± 0.49* 38 (6)	6.60 ± 0.36** 21 (5)
7	14.29 ± 0.60** 46 (7)	8.26 ± 2.09** 15 (3)	12.00 ± 0.60 43 (8)	11.02 ± 0.66* 29 (6)	9.22 ± 0.51** 21 (5)
14	28.11 ± 0.99** 46 (7)	19.38 ± 2.79** 11 (3)	24.20 ± 0.71* 43 (8)	22.68 ± 0.84** 22 (6)	13.52 ± 1.87** 18 (5)
21	39.67 ± 0.95** 46 (7)	34.14 ± 2.16 11 (3)	36.39 ± 1.88 43 (8)	34.57 ± 1.34 22 (6)	22.36 ± 1.93** 16 (4)
28	73.19 ± 1.66** 46 (7)	59.35 ± 4.02* 11 (3)	63.74 ± 2.63* 43 (8)	60.92 ± 1.81** 22 (6)	32.74 ± 3.04** 16 (4)
1–28 ^e	66.09 ± 1.50** 46 (7)	53.69 ± 3.63* 11 (3)	57.43 ± 2.56* 43 (8)	55.11 ± 1.94** 22 (6)	28.04 ± 2.94** 16 (4)
Female					
1	6.56 ± 0.16** 41 (7)	5.41 ± 0.30* 15 (4)	6.14 ± 0.17 49 (8)	5.71 ± 0.27* 31 (6)	4.38 ± 0.36** 18 (4)
4	9.30 ± 0.25** 41 (7)	6.34 ± 0.88* 13 (4)	8.13 ± 0.40 46 (8)	7.49 ± 0.61 31 (6)	5.57 ± 1.03** 16 (4)
7	13.30 ± 0.39** 38 (7)	8.73 ± 1.75* 12 (3)	11.71 ± 0.66 39 (8)	10.90 ± 0.76 28 (6)	7.10 ± 1.60** 16 (4)

Bisphenol AF, NTP DART 08

Postnatal Day ^c	0 ppm	937.5 ppm	1,875 ppm	3,750 ppm	7,500 ppm
14	26.27 ± 0.68** 37 (7)	19.37 ± 3.84* 10 (3)	23.89 ± 0.74 36 (8)	21.67 ± 1.43* 25 (6)	11.63 ± 1.96** 10 (4)
21	37.00 ± 0.83** 37 (7)	32.56 ± 4.86 10 (3)	36.60 ± 1.55 36 (8)	33.07 ± 1.47 25 (6)	17.82 ± 3.28** 9 (4)
28	66.93 ± 0.99** 37 (7)	57.37 ± 5.26 10 (3)	61.59 ± 1.85 36 (8)	57.58 ± 1.94** 25 (6)	24.36 ± 4.58** 9 (4)
1–28 ^e	60.34 ± 0.93** 37 (7)	51.98 ± 4.73 10 (3)	55.43 ± 1.77 36 (8)	51.92 ± 2.06** 25(6)	20.33 ± 4.27** 9 (4)
Male and Female					
1	6.84 ± 0.19** 93 (7)	5.31 ± 0.40** 33 (4)	6.23 ± 0.15 98 (8)	5.77 ± 0.21* 70 (6)	4.61 ± 0.38** 40 (6)
4	9.64 ± 0.31** 92 (7)	5.95 ± 1.02** 30 (4)	8.23 ± 0.36 93 (8)	7.64 ± 0.54* 69 (6)	6.04 ± 0.85** 37 (6)
7	13.89 ± 0.49** 84 (7)	7.44 ± 1.71** 27 (4)	11.84 ± 0.61 82 (8)	10.90 ± 0.71 57 (6)	8.23 ± 1.42** 37 (6)
14	27.33 ± 0.89** 83 (7)	17.42 ± 3.38** 21 (4)	24.04 ± 0.62 79 (8)	21.81 ± 1.28* 47 (6)	12.69 ± 2.43** 28 (6)
21	38.55 ± 0.99** 83 (7)	31.19 ± 4.16 21 (4)	36.46 ± 1.62 79 (8)	33.31 ± 1.35 47 (6)	19.69 ± 3.92** 25 (5)
28	70.60 ± 1.63** 83 (7)	55.54 ± 5.22* 21 (4)	62.72 ± 2.08 79 (8)	58.61 ± 1.92* 47 (6)	28.48 ± 6.48** 25 (5)
1–28 ^e	63.72 ± 1.49** 83 (7)	50.28 ± 4.75* 21 (4)	56.49 ± 2.02 79 (8)	52.87 ± 2.05* 47 (6)	24.20 ± 6.05** 25 (5)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

The 15,000 ppm group was removed on gestation day 10 or 11 due to excessive body weight loss.

^aStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

^bData are displayed as mean ± standard error of the litter means. Body weight data are presented in grams. Changes in n are the result of removing litters with no surviving pups by: postnatal day (PND) 1 (one litter with no surviving male pups in the 937.5 ppm group and one litter with no surviving female pups in the 7,500 ppm group), PND 7 (one litter with no surviving female pups in the 937.5 ppm group), and PND 17 (one litter with no surviving male or female pups in the 7,500 ppm group).

^cAs litters were not standardized, pup weights throughout the entire postnatal period were adjusted using the total live litter size on PND 1.

^dn = the number of pups examined (number of litters).

^eBody weight gain (data are presented in grams).

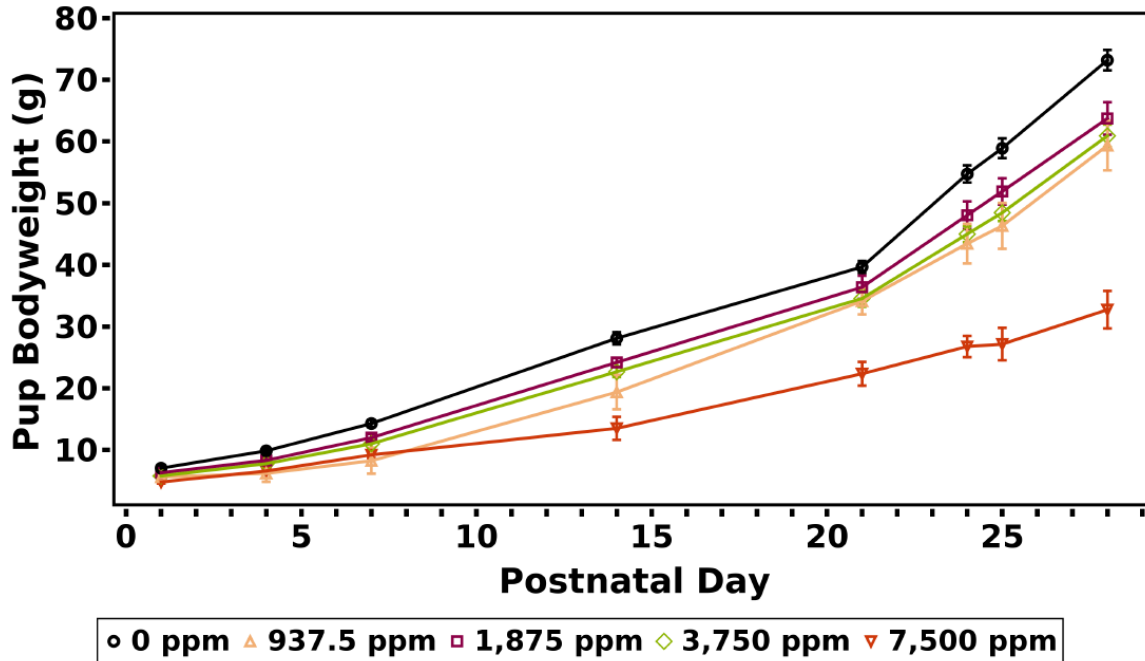


Figure 6. Lactation Growth Curves for F₁ Male Pups Following Perinatal Exposure to Bisphenol AF (Dose Range-finding Study)

Information for statistical significance in male pup weights is provided in Table 8.

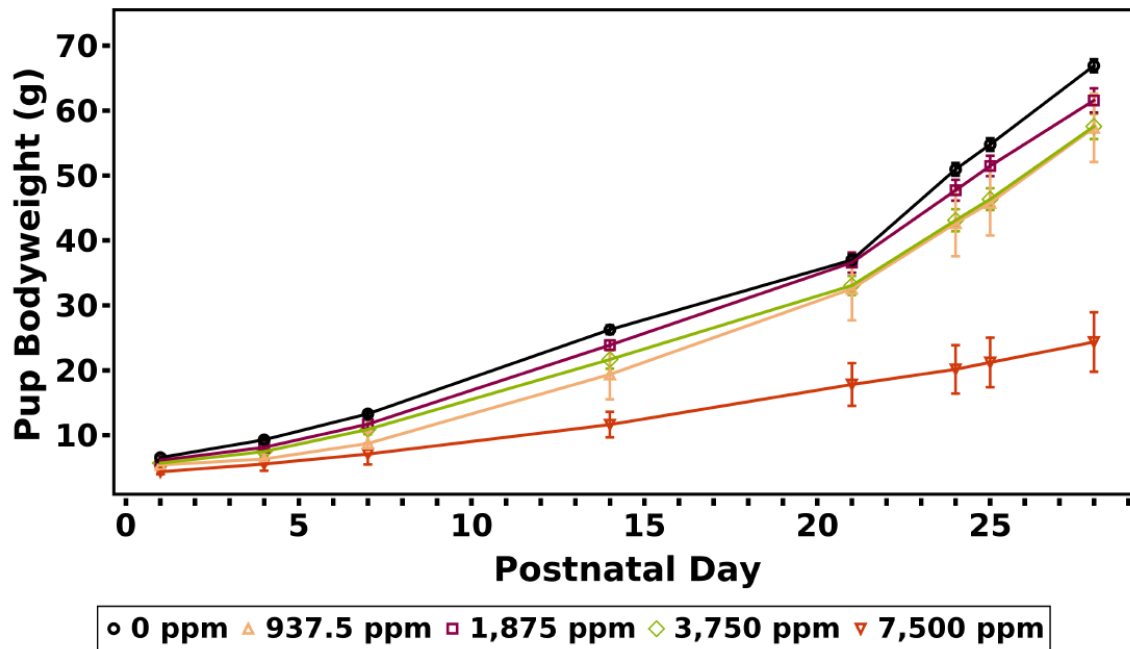


Figure 7. Lactation Growth Curves for F₁ Female Pups Following Perinatal Exposure to Bisphenol AF (Dose Range-finding Study)

Information for statistical significance in female pup weights is provided in Table 8.

Exposure Concentration Selection Rationale for the Modified One-Generation Study of Bisphenol AF

The selection of 3,750 ppm BPAF as the high-exposure concentration was based on maternal and pup toxicity (significantly decreased body weights) observed at 7,500 ppm. Exposure concentration spacing (338, 1,125, and 3,750 ppm) was selected to achieve a no-observed-adverse-effect level and to avoid excessive overlap of the ingested doses due to increased feed consumption during pregnancy.

Modified One-Generation Study

F₀ Generation: Maternal Findings

Maternal effects were evaluated from GD 6 through LD 28, as shown in Figure 8. Viability, clinical observations, gestation and lactation mean body weights, feed consumption, and reproductive performance results are presented below.

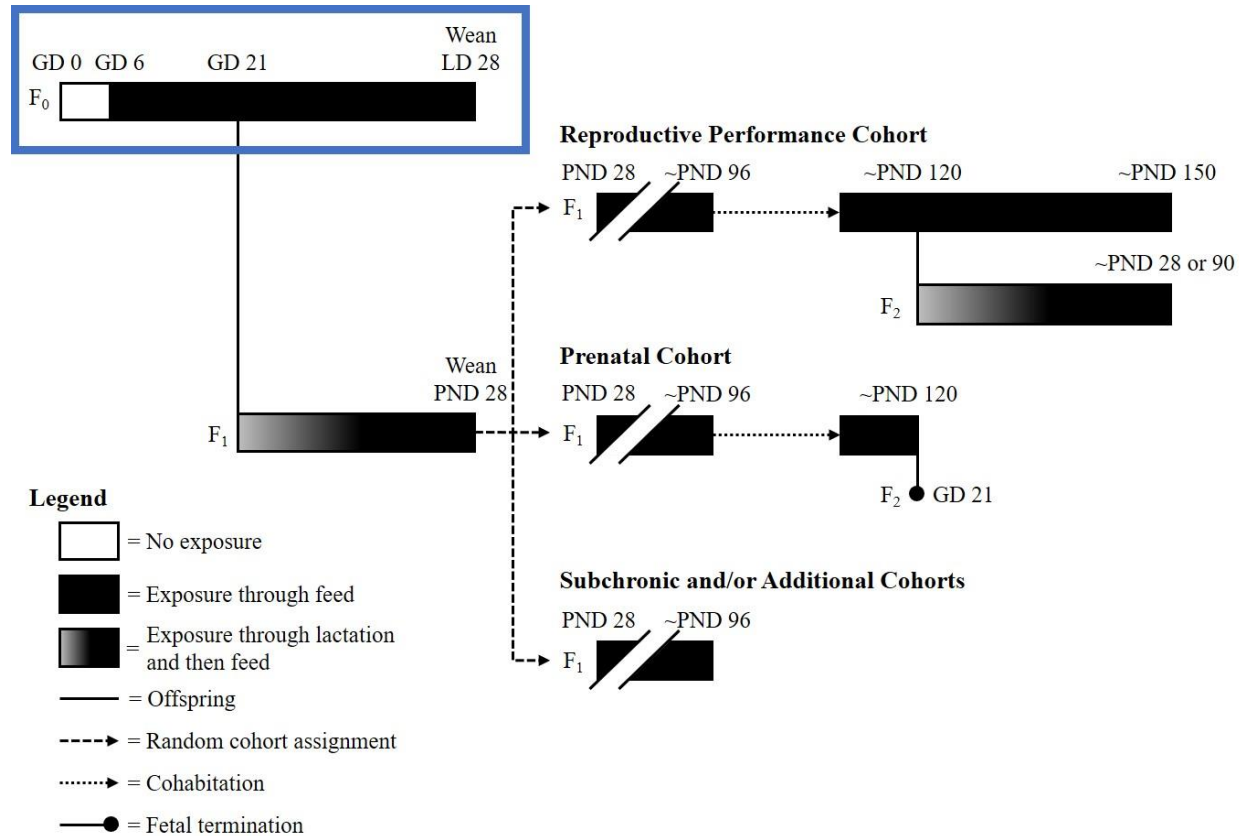


Figure 8. Design of the Modified One-Generation Study – F₀ Generation

GD = gestation day; LD = lactation day; PND = postnatal day.

F₀ Viability and Clinical Observations

BPAF exposure did not affect survival of the F₀ females (Appendix E). No clinical observations were attributed to BPAF exposure during gestation or lactation (Appendix E).

F₀ Gestation Body Weights and Feed Consumption

F₀ females exposed to 3,750 ppm BPAF displayed biologically and statistically significantly decreased gestation body weights, and females exposed to 1,125 and 3,750 ppm showed significantly decreased body weight gains over the GD 6–21 interval (Table 9; Figure 9). On GD 21, the 3,750 ppm female mean body weights were significantly decreased by 13% relative to the control animals, and mean body weight gain over the GD 6–21 interval for the 1,125 and 3,750 ppm BPAF groups were significantly decreased by 21 and 40%, respectively, compared to the weight gain of the control group (Table 9). Consistent with observations in the dose range-

finding study, there was a significant decrease in body weight gain at the beginning of the study at the higher exposure concentrations (1,125 and 3,750 ppm) and likely reflects lower palatability of the dosed feed. The significant decreases in mean body weight gain were sustained throughout most of gestation (GD 6–21) for both the 1,125 and 3,750 ppm groups. There were no effects of BPAF exposure on F₀ female body weights during gestation in the 338 ppm group. There was no reduction in litter size on LD 0 in the BPAF-exposed groups; however, there was a significant decrease of LD 1 pup weights of 9% and 15% for the 1,125 and 3,750 ppm groups, respectively (Appendix E). This observation suggests that the lower relative maternal mean body weights could be due to an effect on the collective weight of the uterine contents.

Significant variability in feed consumption was observed across the intervals in the 1,125 and 3,750 ppm groups (Table 10). Although the higher feed consumption values likely represented feed wastage, there was a negative trend for feed consumption for select intervals by the 1,125 and 3,750 ppm groups that corresponds with similar intervals showing significant decreases in mean body weight gain. BPAF intakes for F₀ females in the 338, 1,125, and 3,750 ppm groups, based on feed consumption and dietary concentrations for GD 6–21, were approximately 24, 81, and 279 mg/kg/day, respectively.

Table 9. Summary of Mean Body Weights and Body Weight Gains of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation

Parameter ^{a,b}	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Gestation Body Weight				
Gestation Day				
6	238.2 ± 1.9 (30)	239.3 ± 2.5 (32)	237.1 ± 2.1 (33)	237.6 ± 2.1 (29)
9	250.8 ± 2.2** (30)	250.2 ± 2.6 (32)	240.4 ± 2.0** (33)	233.8 ± 2.2** (29)
12	264.8 ± 2.5** (30)	265.0 ± 2.9 (32)	250.6 ± 2.2** (33)	240.9 ± 2.4** (29)
15	283.0 ± 3.2** (30)	282.9 ± 3.3 (32)	266.7 ± 2.4** (33)	251.8 ± 2.8** (29)
18	318.6 ± 5.1** (30)	320.6 ± 4.4 (32)	303.4 ± 2.9** (33)	284.3 ± 3.5** (29)
21 ^c	356.8 ± 7.6** (27)	359.7 ± 5.9 (29)	333.2 ± 3.8** (30)	309.9 ± 4.4** (26)
Gestation Weight Change				
Gestation Day Interval				
6–21 ^c	119.3 ± 7.0** (27)	120.6 ± 4.2 (29)	94.8 ± 2.9** (30)	71.9 ± 4.1** (26)
3–6	13.4 ± 0.8 (30)	15.0 ± 1.0 (32)	15.6 ± 1.0 (33)	13.7 ± 0.9 (29)
6–9	12.6 ± 0.5** (30)	10.9 ± 0.8 (32)	3.3 ± 0.8** (33)	-3.8 ± 0.9** (29)
9–12	14.0 ± 0.8** (30)	14.8 ± 0.8 (32)	10.1 ± 0.9** (33)	7.1 ± 1.5** (29)
12–15	18.2 ± 1.4** (30)	17.9 ± 1.0 (32)	16.1 ± 0.9 (33)	11.0 ± 1.0** (29)
15–18	35.6 ± 2.5** (30)	37.7 ± 1.5 (32)	36.7 ± 1.3 (33)	32.5 ± 1.7 (29)
18–21	40.1 ± 2.7** (27)	39.3 ± 1.8 (29)	29.2 ± 1.6** (30)	26.3 ± 1.6** (26)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

^aData are displayed as mean ± standard error (n); body weight data are presented in grams.

^bStatistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^cThree dams were removed in each group on gestation day 18 for biological sample collection.

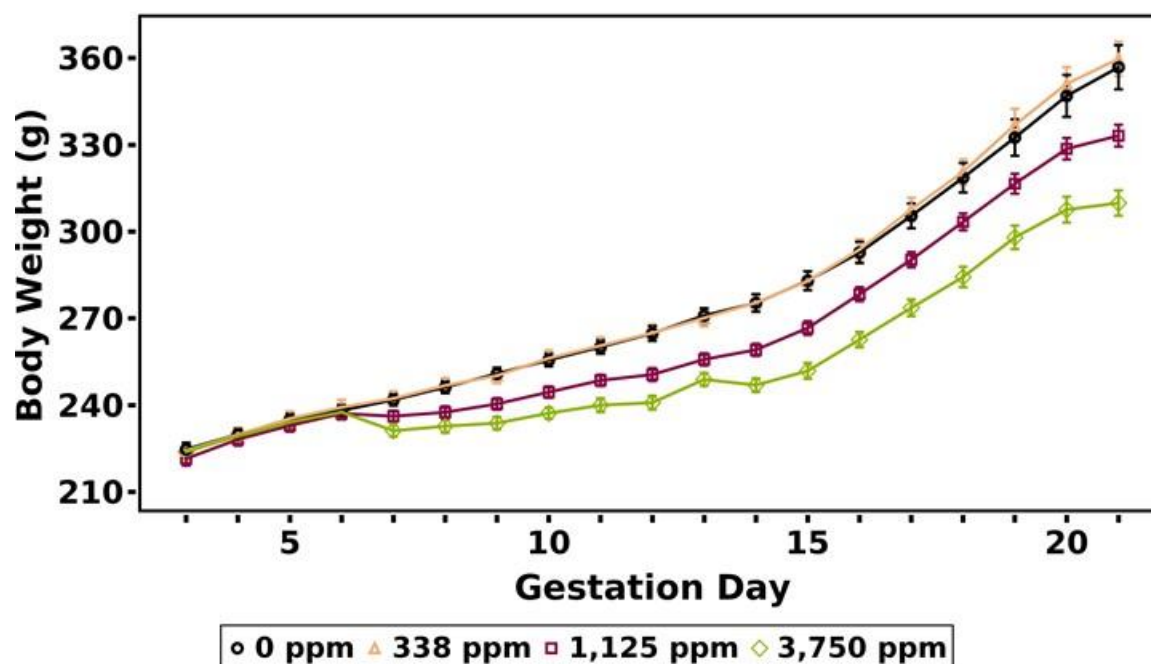


Figure 9. Growth Curves for F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation

Information for statistical significance in maternal weights is provided in Table 9.

Table 10. Summary of Feed and Test Article Consumption of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation

Gestation Day Interval ^{a,b}	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Feed Consumption (g/animal/day)^{c,d}				
6–21	20.0 ± 0.3* (27)	20.5 ± 0.4 (29)	19.5 ± 0.7 (30)	19.2 ± 0.5 (26)
3–6	17.7 ± 0.2 (30)	18.2 ± 0.3 (32)	18.2 ± 0.3 (33)	17.7 ± 0.3 (29)
6–9	18.1 ± 0.3* (30)	18.1 ± 0.3 (31)	15.2 ± 0.7** (27)	20.6 ± 2.9 (7)
9–12	18.8 ± 0.3** (29)	18.8 ± 0.3 (32)	16.4 ± 0.7** (32)	15.5 ± 1.1** (29)
12–15	19.5 ± 0.3 (30)	19.9 ± 0.5 (32)	23.9 ± 1.6 (29)	26.8 ± 5.3 (3)
15–18	22.4 ± 0.6** (30)	23.1 ± 0.4 (32)	20.6 ± 0.5** (33)	18.6 ± 0.6** (26)
18–21 ^e	22.0 ± 0.5 (27)	22.1 ± 0.5 (29)	19.1 ± 0.6** (30)	23.5 ± 1.1 (18)
Chemical Intake (mg/kg/day)^{f,g}				
6–21	0.0 ± 0.0 (27)	24.3 ± 0.3 (29)	80.9 ± 2.6 (30)	278.7 ± 7.8 (26)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData are displayed as mean ± standard error (n), where n = the number of dams. Feed consumption is not reported for nonpregnant animals during the gestation phase.

^bFor each dam, calculation of consumption values for the GD 6–21 interval was performed using all valid data for the animal, even if data were unavailable for some of the subintervals.

^cFeed consumption values were excluded when excessive spillage was recorded. One value was removed as an outlier for gestation day (GD) 12–15 from the 1,125 ppm group.

^dStatistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

^eThree dams were removed in each group on GD 18 for biological sample collection.

^fChemical intake calculated as: $([\text{exposure concentration} \times \text{feed consumption}]/[\text{average body weight of day range}])$.

^gNo statistical analysis performed on the chemical intake data.

Maternal Reproductive Performance

Across all exposure groups, 16 out of 140 time-mated female rats were not pregnant: 5 in the control group, 3 in the 338 ppm group, 2 in the 1,125 ppm group, and 6 in the 3,750 ppm group (Table 11). There was no effect of BPAF exposure on the proportion of dams that produced viable litters; however, there was a slight but significant increase in gestation length for F₀ dams in the 3,750 ppm group. There was no effect of BPAF exposure on initial mean litter size or sex ratio; however, LD 1 pup mean body weights were lower by 9% and 15% when compared to the control pups for the 1,125 and 3,750 ppm groups, respectively (Table 11).

Table 11. Summary of the Reproductive Performance of F₀ Female Rats Exposed to Bisphenol AF in Feed during Gestation

Parameter ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Time-mated Females (GD 6)	35	35	35	35
Females Pregnant (%)	30 (85.7)	32 (91.4)	33 (94.3)	29 (82.9)
Females Not Pregnant (%)	5 (14.3)	3 (8.6)	2 (5.7)	6 (17.1)
Dams Removed on GD 18 ^b	3	3	3	3
Dams Not Delivering with Evidence of Pregnancy (%)	2 (7.41)	1 (3.44)	1 (3.33)	1 (3.85)
Dams with Litters on LD 0 (%) ^c	25 (92.6)	28 (96.6)	29 (96.7)	25 (96.2)
Gestation Length (days) ^{d,e,f}	22.1 ± 0.1** (25)	22.2 ± 0.1 (28)	22.1 ± 0.1 (29)	22.4 ± 0.1** (25)
Live Litter Size on LD 0 ^{d,f}	13.2 ± 0.4 (24) ^g	11.9 ± 0.6 (28)	12.9 ± 0.4 (28) ^g	12.5 ± 0.5 (24) ^g
LD 1 Pup Weight ^{f,h,i}	6.68 ± 0.07** 314 (24)	6.40 ± 0.16 319 (28)	6.06 ± 0.11** 354 (28)	5.65 ± 0.16** 263 (23) ^g
Percent Live Male Pups per Litter ^{d,f}	43.01 ± 2.58 (24)	50.64 ± 3.01 (28)	49.27 ± 2.78 (28)	50.58 ± 3.20 (24)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

GD = gestation day; LD = lactation day.

^aAnimals removed from the study between mating and littering were excluded from calculations of % littered females.

^bDams were removed on GD 18 for biological sample collection.

^cPercentage is the number of littered females/pregnant females. Statistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.

^dStatistical analysis was performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

^eGestation length calculated for sperm-positive females that delivered a litter.

^fData are displayed as mean ± standard error (n).

^gChanges in n are the result of removing litters with no surviving pups by: LD 0 (one litter in the vehicle control group, one litter in the 1,125 ppm group, and one litter in the 3,750 ppm group) and LD 1 (one litter in the 3,750 ppm group).

^hn = the number of pups examined (number of litters).

ⁱStatistical analysis performed using mixed models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

Lactation Body Weights and Feed Consumption

F₀ females in the 1,125 and 3,750 ppm BPAF groups displayed significant exposure concentration-dependent decreases in mean body weights during most of the lactation period (Table 12; Figure 10). There were no effects of BPAF exposure on F₀ female body weights during lactation in the 338 ppm group.

Bisphenol AF, NTP DART 08

Feed consumption during the LD 1–13 interval was higher for both mean absolute (g/animal/day) and relative (g/kg/day) feed consumption by the 1,125 and 3,750 ppm groups, compared to the control group, with relative consumption significantly increased. Maternal feed consumption during lactation by the 338 ppm group was similar to the control group. BPAF intakes by F₀ females, based on feed consumption and dietary concentrations for LD 1–13, were 57, 223, and 852 mg/kg/day for the 338, 1,125, and 3,750 ppm groups, respectively (Table 12).

Table 12. Summary of Mean Body Weights, Body Weight Gains, and Feed and Test Article Consumption of F₀ Female Rats Exposed to Bisphenol AF in Feed during Lactation^a

Lactation Day	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Body Weight (g)^b				
1	258.2 ± 3.1** (24)	257.8 ± 3.8 (28)	225.5 ± 2.9** (28)	213.9 ± 3.0** (24)
10	298.0 ± 2.8** (23) ^c	294.0 ± 4.0 (24) ^{c,d}	272.3 ± 3.4** (25) ^d	253.6 ± 3.0** (23) ^c
13	306.5 ± 2.3** (23)	304.1 ± 3.7 (24)	275.6 ± 3.4** (25)	255.3 ± 2.5** (22) ^c
16	306.5 ± 2.5** (23)	303.1 ± 3.7 (24)	282.8 ± 3.0** (25)	267.7 ± 2.8** (22)
28	279.9 ± 3.3 (23)	273.7 ± 3.1 (24)	270.3 ± 3.0 (25)	274.4 ± 3.0 (22)
Body Weight Gain (g)^b				
4–28	1.7 ± 2.5** (23)	–1.6 ± 2.6 (24)	25.4 ± 2.5** (25)	41.6 ± 2.8** (22)
Feed Consumption^e				
1–13 (g/animal/day)	49.0 ± 0.7 (23)	47.6 ± 1.1 (24)	50.7 ± 1.4 (25)	54.4 ± 2.3 (22)
1–13 (g/kg/day)	172.5 ± 2.6** (23)	168.7 ± 3.8 (24)	198.3 ± 5.3** (25)	227.3 ± 9.2** (22)
Chemical Intake (mg/kg/day)^{f,g}				
1–13	0.0 ± 0.0 (23)	57.0 ± 1.3 (24)	223.1 ± 6.0 (25)	852.3 ± 34.5 (22)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

^aData are displayed as mean ± standard error (n), where n = the number of dams. Feed consumption values were excluded when excessive spillage was recorded.

^bStatistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^cDams with whole litter loss were removed on lactation day (LD) 1 (one dam in the 3,750 ppm group), LD 4 (one dam in the vehicle control group and one dam in the 338 ppm group), and LD 10 (one dam in the 3,750 ppm group).

^dThree dams were removed on LD 4 from the 338 and 1,125 ppm groups for biological sample collection.

^eStatistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

^fChemical intake calculated as: $([\text{exposure concentration} \times \text{feed consumption}]/[\text{average body weight of day range}])$.

^gNo statistical analysis performed on the chemical intake data.

Bisphenol AF, NTP DART 08

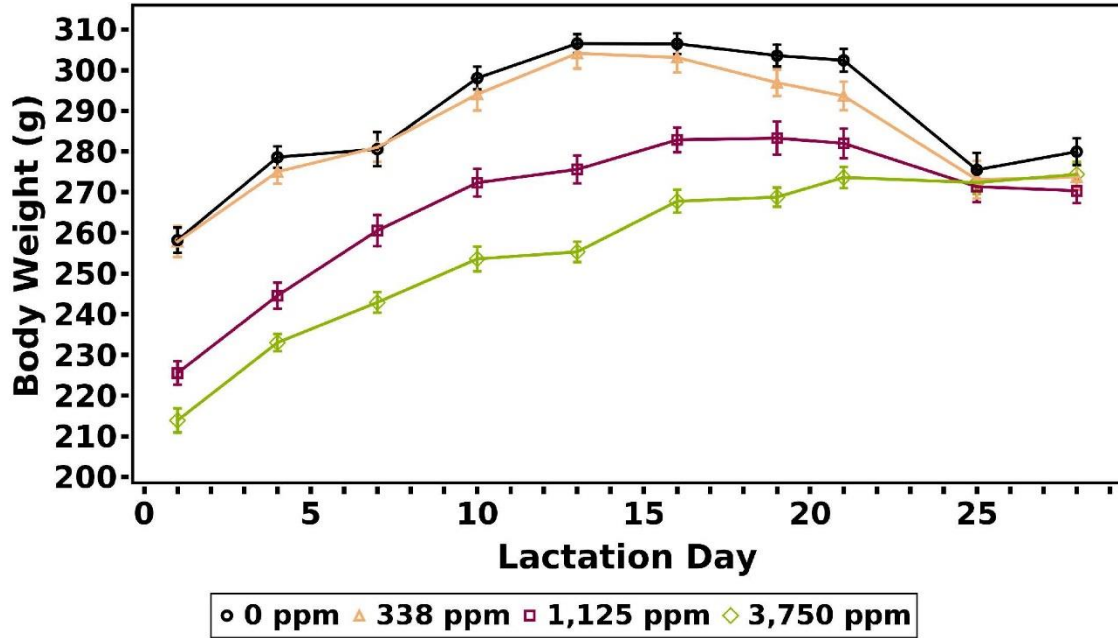


Figure 10. Growth Curves for F₀ Female Rats Exposed to Bisphenol AF in Feed during Lactation

Information for statistical significance in maternal weights is provided in Table 12.

F₁ Generation: Prewearing

F₁ male and female rats were evaluated during the preweaning period from PND 0 through PND 28, as shown in Figure 11. Viability, clinical observations, and mean body weight results are presented below.

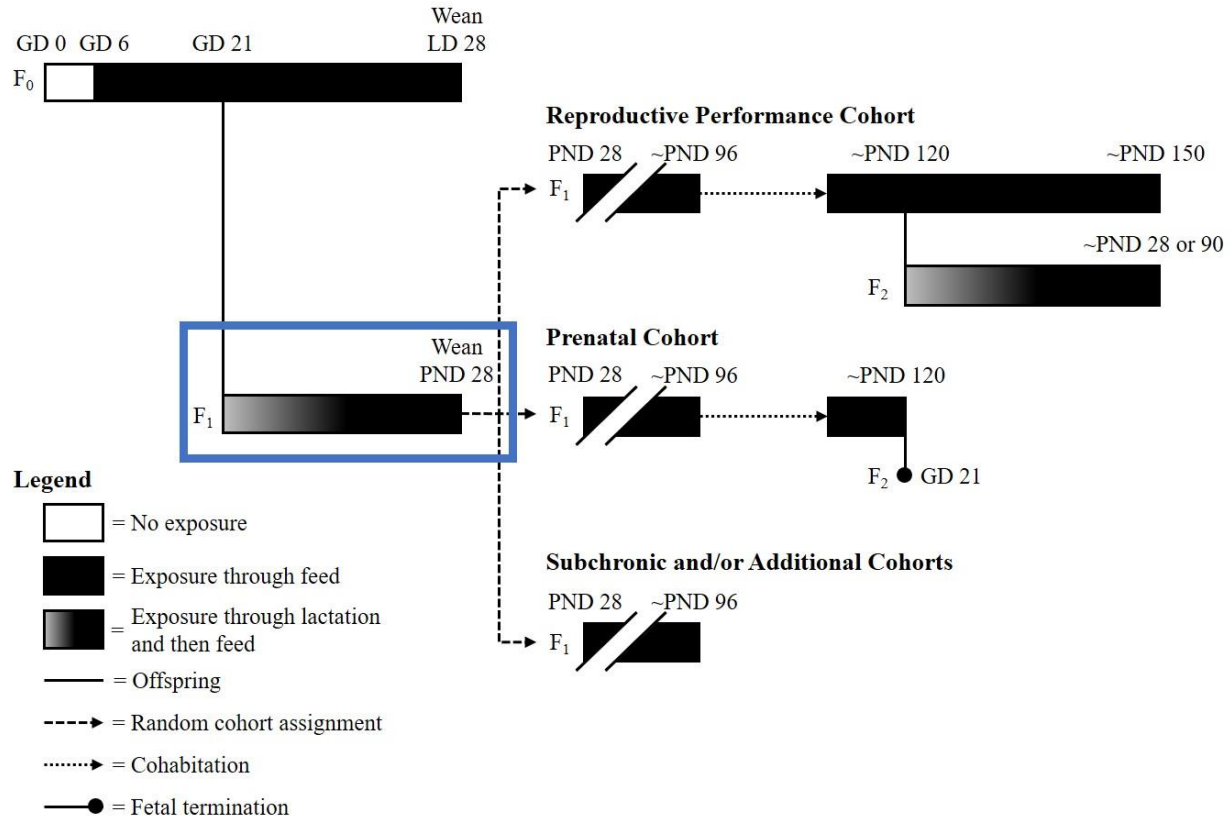


Figure 11. Design of the Modified One-Generation Study – F₁ Generation: Prewearing

GD = gestation day; LD = lactation day; PND = postnatal day.

F₁ Viability and Clinical Observations

There was a significant decrease in pup survival in the 3,750 ppm group over the PND 1–4 interval only; all other intervals were unaffected by BPAF exposure relative to the control group (Table 13; Appendix E). The mean number of live pups per litter was significantly decreased in the 3,750 ppm group on PNDs 4 (prestandardization) and 7 relative to the control group. Mean live litter size on PNDs 1 and 4 was lower by approximately two pups, with the total number of dead pups per litter significantly increased over the PND 1–4 interval in the 3,750 ppm group relative to the control group. Of note, one control female had a litter that did not survive through PND 4, and three females in the 3,750 ppm group had no live pups on PND 1 or low pup viability, resulting in their removal by PND 4. There was no effect of BPAF exposure on mean sex ratio (Table 11).

Clinical observations associated with BPAF exposure occurred in the 3,750 ppm group and were limited to yellow stained fur, which was observed in nine individual female pups across five litters from PND 21 through PND 28. Clinical observations noted in individual pups from all

Bisphenol AF, NTP DART 08

exposure groups, including the control group, were typically indicative of an individual pup not thriving (e.g., no milk in stomach, cold to touch). Other findings observed, including sores, swelling, alopecia, tail damage, and nasal discharge, were limited to a few pups or were only observed in the control group.

Table 13. Summary of F₁ Litter Size and Pup Survival Following Perinatal Exposure to Bisphenol AF

Postnatal Day	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. of Live Pups (Litters)^a				
0	316 (25)	333 (28)	361 (29)	301 (25)
Total Litter Size^{b,c}				
0	13.2 ± 0.7 (25)	13.2 ± 0.6 (28)	13.4 ± 0.3 (29)	13.2 ± 0.4 (25)
Live Litter Size^{b,c}				
0	13.2 ± 0.4 (24) ^d	11.9 ± 0.6 (28)	12.9 ± 0.4 (28) ^d	12.5 ± 0.5 (24) ^d
1	13.0 ± 0.4 (24)	11.4 ± 0.7 (28)	12.6 ± 0.4 (28)	11.4 ± 0.7 (23) ^d
4 (prestandardization)	13.1 ± 0.4* (23) ^d	11.6 ± 0.6 (27) ^d	12.4 ± 0.4 (28)	10.6 ± 0.8** (23)
4 (poststandardization)	9.8 ± 0.2 (23)	9.5 ± 0.3 (24) ^e	10.0 ± 0.0 (25) ^e	9.0 ± 0.5 (23)
7	9.8 ± 0.2* (23)	9.4 ± 0.3 (24)	9.7 ± 0.1 (25)	8.8 ± 0.5* (23)
13	9.7 ± 0.2* (23)	9.2 ± 0.4 (24)	9.3 ± 0.3 (25)	8.8 ± 0.4 (22) ^d
21	9.7 ± 0.2 (23)	9.1 ± 0.4 (24)	9.3 ± 0.3 (25)	8.6 ± 0.4 (22)
28	9.7 ± 0.2 (23)	9.0 ± 0.4 (24)	9.2 ± 0.3 (25)	8.6 ± 0.4 (22)
No. of Dead Pups (Litters)^a				
0	13 (12)	37 (10)	27 (12)	29 (14)
1–4	15 (3)	20 (9)	13 (8)	58 (12)
5–28	3 (3)	9 (3)	16 (7)	16 (9)
Dead/Litter^{b,c}				
0	0.52 ± 0.12 (25)	1.32 ± 0.58 (28)	0.93 ± 0.36 (29)	1.16 ± 0.48 (25)
1–4	0.63 ± 0.50* (24) ^d	0.71 ± 0.29 (28)	0.46 ± 0.17 (28) ^d	2.42 ± 0.88* (24) ^d
5–28	0.13 ± 0.07* (23) ^d	0.38 ± 0.29 (24) ^{d,e}	0.64 ± 0.26 (25) ^e	0.70 ± 0.22 (23) ^d
Survival Ratio^{b,c}				
0	0.92 ± 0.04 (25)	0.92 ± 0.03 (28)	0.92 ± 0.04 (29)	0.91 ± 0.04 (25)
1–4	0.95 ± 0.04* (24) ^d	0.92 ± 0.04 (28)	0.96 ± 0.01 (28) ^d	0.82 ± 0.06* (24) ^d
5–28	0.99 ± 0.01* (23) ^d	0.96 ± 0.03 (24) ^{d,e}	0.94 ± 0.03 (25) ^e	0.88 ± 0.05 (23) ^d

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^an = the number of pups examined (number of litters). For no. of dead pups, n is the number of litters contributing dead pups.

^bData are displayed as mean ± standard error of the litter means (n), where n = the number of litters. For F₁ pups, data are displayed as the mean of litter values ± standard error (n) of litter values (number of litters produced by F₀ dams).

^cF₁ litter size and survival endpoints were analyzed using the Jonckheere (trend) and Shirley or Dunn tests (pairwise comparisons). All calculations were based on the last litter observation of the day.

^dChanges in n are the result of removing litters with no surviving pups by: postnatal day (PND) 0 (one litter in the vehicle control group, one litter in the 1,125 ppm group, and one litter in the 3,750 ppm group), PND 1 (one litter in the 3,750 ppm group), PND 2 (one litter in the 338 ppm group), PND 4 (one litter in the vehicle control group), and PND 10 (one litter in the 3,750 ppm group).

^eDecreased number of litters at PND 4 in the 338 and 1,125 ppm groups reflects the animals removed for biological sample collection.

F₁ Body Weights

Male Pups

Male pup mean body weights were significantly decreased throughout the lactation period, with weights 12% and 30% less than the control group on PND 28 for the 1,125 and 3,750 ppm groups, respectively (Table 14; Figure 12).

Female Pups

Female pup mean body weights were also significantly decreased for both the 1,125 and 3,750 ppm groups throughout the lactation period compared to the control group. Pup weights were 9% and 27% less than the control group on PND 28 for the 1,125 and 3,750 ppm groups, respectively (Table 14; Figure 13).

Table 14. Summary of F₁ Male and Female Pup Mean Body Weights and Body Weight Gains Following Perinatal Exposure to Bisphenol AF^{a,b}

Postnatal Day	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Male				
1	6.82 ± 0.07** 135 (24) ^c	6.58 ± 0.17 168 (28)	6.18 ± 0.12** 178 (28)	5.84 ± 0.19** 136 (23)
4 ^d	9.65 ± 0.23** 135 (24)	9.70 ± 0.20 164 (27) ^e	8.53 ± 0.18** 175 (28)	7.74 ± 0.32** 125 (23)
7	14.43 ± 0.32** 104 (23) ^e	14.38 ± 0.41 118 (24) ^f	12.48 ± 0.35** 117 (25) ^f	11.93 ± 0.46** 106 (23)
13	27.57 ± 0.47** 102 (23)	26.77 ± 0.73 115 (24)	24.08 ± 0.56** 111 (25)	21.55 ± 0.56** 104 (22) ^e
28	77.54 ± 1.30** 102 (23)	76.34 ± 1.38 113 (24)	68.49 ± 1.31** 111 (25)	53.94 ± 1.48** 102 (22)
4–28 ^g	67.77 ± 1.16** 102 (23)	66.60 ± 1.29 113 (24)	59.79 ± 1.18** 111 (25)	45.82 ± 1.32** 102 (22)
Female				
1	6.57 ± 0.07** 179 (24)	6.34 ± 0.12 151 (27) ^e	5.98 ± 0.11** 176 (28)	5.56 ± 0.12** 127 (22) ^e
4 ^d	9.17 ± 0.22** 177 (24)	9.15 ± 0.19 149 (27)	8.40 ± 0.19* 173 (28)	7.57 ± 0.19** 118 (22)
7	13.79 ± 0.29** 121 (23) ^e	13.52 ± 0.39 108 (24) ^f	12.46 ± 0.35* 125 (25) ^f	11.28 ± 0.41** 96 (22)
13	26.18 ± 0.53** 121 (23)	25.70 ± 0.63 105 (24)	23.49 ± 0.52** 122 (25)	20.75 ± 0.53** 90 (22)
28	71.32 ± 1.34** 120 (23)	69.14 ± 1.13 104 (24)	64.65 ± 1.31** 119 (25)	51.92 ± 1.33** 88 (22)
4–28 ^g	62.05 ± 1.21** 120 (23)	59.93 ± 1.00 104 (24)	56.09 ± 1.18** 119 (25)	44.15 ± 1.18** 88 (22)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons. Pup weights were adjusted for covariate litter size: total live on postnatal day (PND) 1 for day 1 through day 4 and number of live pups poststandardization for later days.

^bData are displayed as mean ± standard error of the litter means. Body weights are presented in grams.

^cn = the number of pups examined (number of litters).

^dPND 4 weights are prestandardization.

^eChanges in n are the result of removing litters with no surviving pups by: PND 1 (one litter with no surviving female pups in the 338 ppm group and one litter with no surviving female pups in the 3,750 ppm group), PND 2 (one litter with no surviving male pups in the 338 ppm group), PND 4 (one litter in the vehicle control group, after pups were weighed on PND 4), and PND 10 (one litter with no surviving male pups in the 3,750 ppm group).

^fDecreased number of litters at PND 7 in the 338 and 1,125 ppm groups reflects the animals removed at PND 4 for biological sample collection.

^gBody weight gain (data are presented in grams).

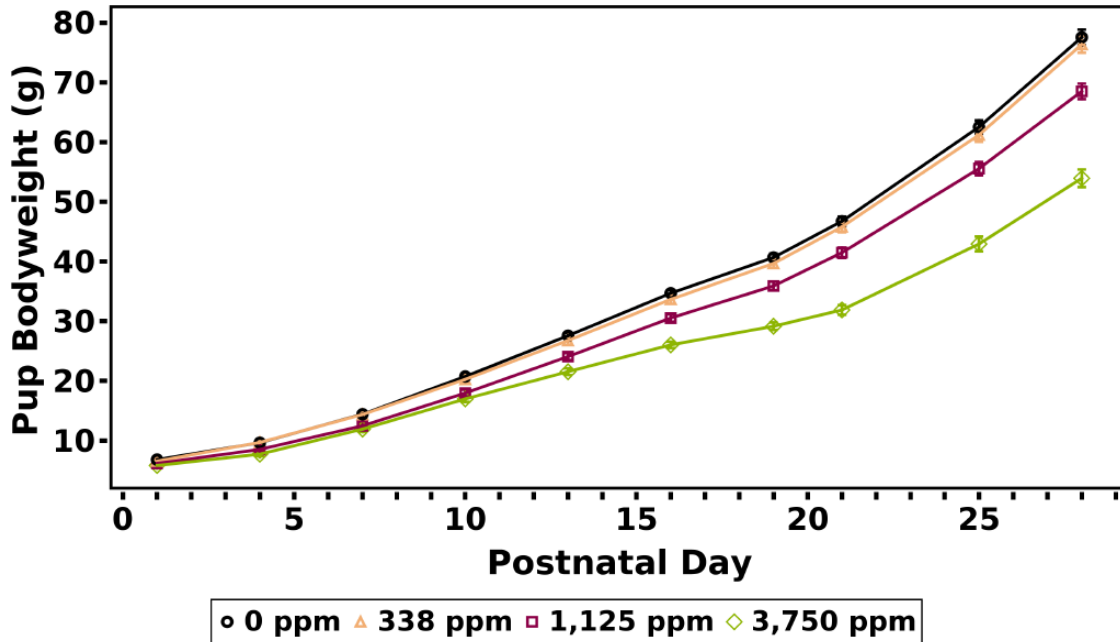


Figure 12. Lactation Growth Curves for F₁ Male Pups Following Perinatal Exposure to Bisphenol AF

Information for statistical significance in male pup weights is provided in Table 14.

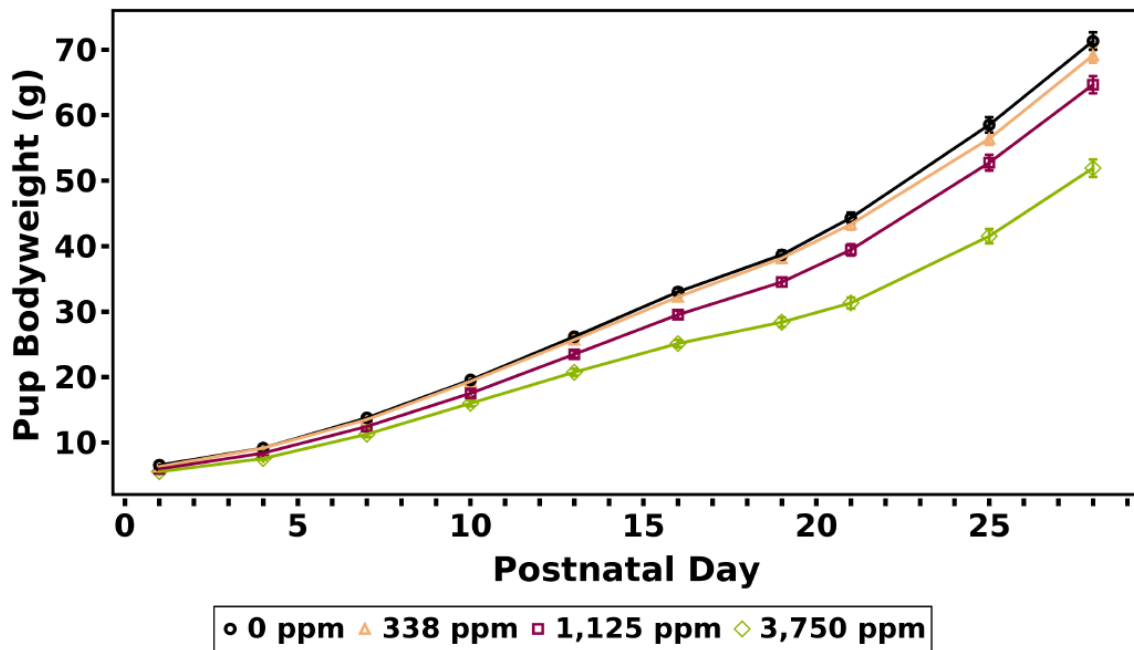


Figure 13. Lactation Growth Curves for F₁ Female Pups Following Perinatal Exposure to Bisphenol AF

Information for statistical significance in female pup weights is provided in Table 14.

F₀ Necropsy

F₀ dams were necropsied on LD 28 following pup weaning, when the F₀ dams were approximately 19 weeks of age. There were no BPAF-related gross or microscopic findings in the F₀ females. Gross findings in the dams at scheduled necropsy were limited to singular incidences or were not exposure related (e.g., one control female with an ovarian cyst, two females at 338 ppm with a hepatodiaphragmatic nodule, and one female at 3,750 ppm with a thickened uterus) (Appendix E). Microscopic findings were limited to confirmation of gross findings.

F₁ Generation: Postweaning through Sexual Maturity

F₁ male and female rats were evaluated from postweaning through sexual maturity, as shown in Figure 14. Viability, clinical observations, mean body weights, feed consumption, and developmental endpoint results are presented below.

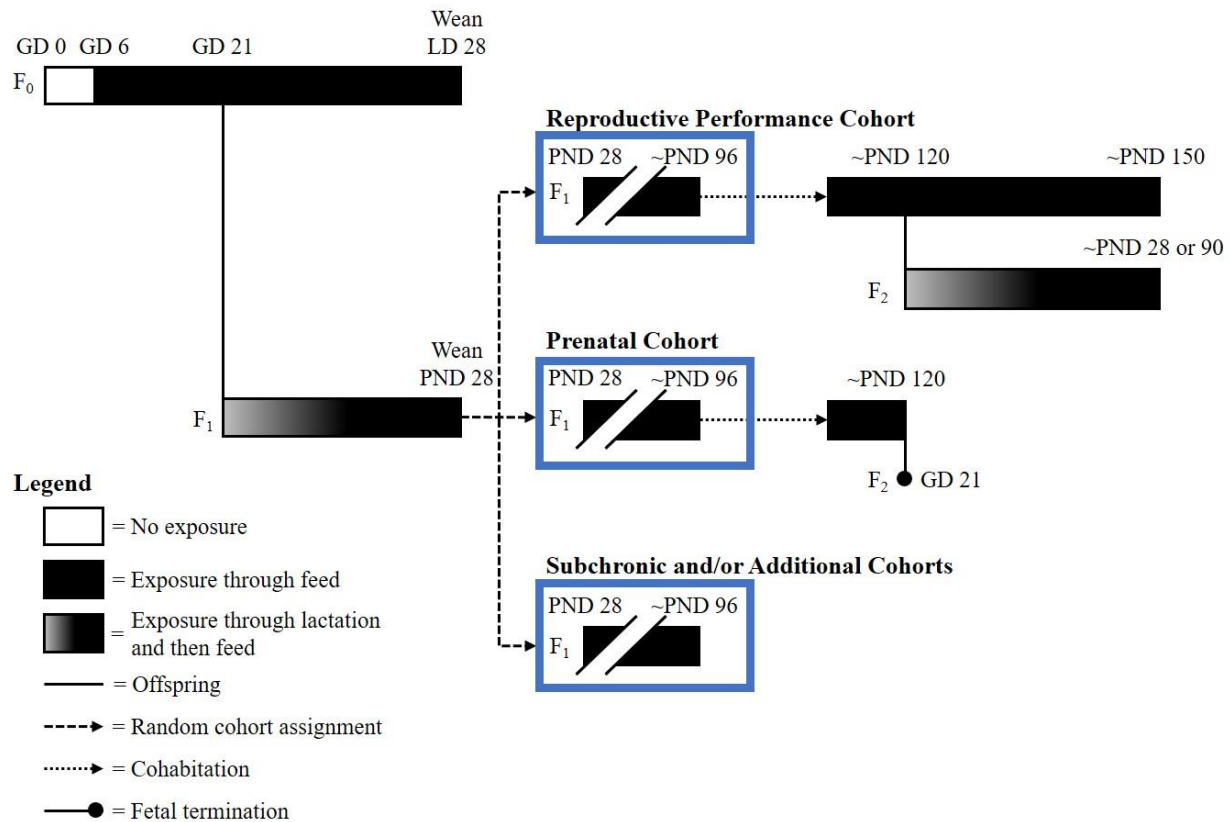


Figure 14. Design of the Modified One-Generation Study – F₁ Generation: Postweaning

GD = gestation day; LD = lactation day; PND = postnatal day.

F₁ Viability and Clinical Observations

There was no effect of BPAF exposure on the survival of F₁ males or females, with the exception of two females in the 3,750 ppm group that were euthanized due to malformations of the vagina (no apparent vaginal opening) (Appendix E). Three additional unscheduled deaths were recorded but were not deemed related to BPAF exposure (one pup in the 3,750 ppm group sustained tail damage during a cage change, one pup in the control group was found moribund, and one pup in

the 338 ppm group was found dead with necropsy findings of nodules on the liver and spleen and diagnosed as having malignant leukemia).

Clinical observations of small testis or missing testis was noted in 26 and 17 male pups, respectively, in the 3,750 ppm group. No BPAF-related clinical observations were noted in the F₁ female pups. All other clinical observations noted were across all exposure groups, including the control groups, on a sporadic basis (Appendix E).

F₁ Body Weights and Feed Consumption

Males (Postweaning)

Mean body weights between PND 28 and PND 98 were significantly decreased in males in the 1,175 and 3,750 ppm groups (Table 15; Figure 15). Male mean body weights for the 1,125 and 3,750 ppm groups were lower by 11% and 30% relative to the control group, respectively, on PND 28 and lower by 13% and 38% relative to the control group, respectively, on PND 98. Mean body weights for F₁ males in the 338 ppm group were $\geq 95\%$ of the control group from PND 28 through PND 98.

There was a significant decrease in absolute feed consumption (g/animal/day) over the PND 28–98 interval by the F₁ males in the 1,125 and 3,750 ppm groups (Table 15); however, this might have resulted from the reduced size of the pups in these groups given that relative feed consumption values (g/kg/day) were similar to—or significantly increased compared to—the control group. Feed consumption by F₁ males in the 338 ppm group was similar to the control group—although significant decreases in absolute feed consumption were observed for some time intervals, overall feed consumption during the postweaning period was similar to the control group (Appendix E).

BPAF intakes by F₁ males, based on feed consumption and dietary concentrations for PND 28–98, were 28, 98, and 411 mg/kg/day at 338, 1,125, and 3,750 ppm, respectively.

Table 15. Summary of Postweaning Mean Body Weights, Body Weight Gains, and Feed and Test Article Consumption of All F₁ Male Rats Exposed to Bisphenol AF in Feed

Postnatal Day ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Body Weight (g)^{b,c}				
28	76.3 ± 1.4** 54 (22)	75.5 ± 1.5 55 (24)	67.6 ± 1.4** 53 (22)	53.6 ± 1.6** 50 (20)
91	376.0 ± 3.9** 54 (22)	362.4 ± 4.8 54 (24)	325.4 ± 4.8** 53 (22)	231.2 ± 4.4** 50 (20)
98	386.3 ± 4.4** 54 (22)	373.7 ± 5.2 54 (24)	334.4 ± 5.0** 53 (22)	238.3 ± 4.6** 50 (20)
105	397.0 ± 4.0** 54 (22)	383.0 ± 5.3 54 (24)	344.0 ± 5.0** 53 (22)	245.0 ± 4.4** 50 (20)
Body Weight Gain (g)^{b,c}				
28–91	299.7 ± 3.0** 54 (22)	286.9 ± 4.4 54 (24)	257.8 ± 4.1** 53 (22)	177.5 ± 4.0** 50 (20)

Bisphenol AF, NTP DART 08

Postnatal Day ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Postweaning Feed Consumption^{d,e}				
28–98 (g/animal/day)	21.8 ± 0.2** (25)	21.3 ± 0.4 (26)	19.8 ± 0.3** (24)	18.5 ± 0.3** (25)
28–98 (g/kg/day)	80.3 ± 0.5** (25)	81.9 ± 0.9 (26)	86.7 ± 1.4** (24)	109.5 ± 2.2** (25)
Chemical Intake (mg/kg/day)^{e,f,g}				
28–98	0.0 ± 0.0 (25)	27.7 ± 0.3 (26)	97.5 ± 1.5 (24)	410.6 ± 8.2 (25)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

^aData are displayed as mean ± standard error (n). Feed consumption values were excluded when excessive spillage was recorded.

^bStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple comparisons.

^cn = the number of pups examined (number of litters).

^dStatistical analysis performed using the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

^en = number of cages.

^fChemical intake calculated as: $([\text{exposure concentration} \times \text{feed consumption}]/[\text{average body weight of day range}])$.

^gNo statistical analysis performed on the chemical intake data.

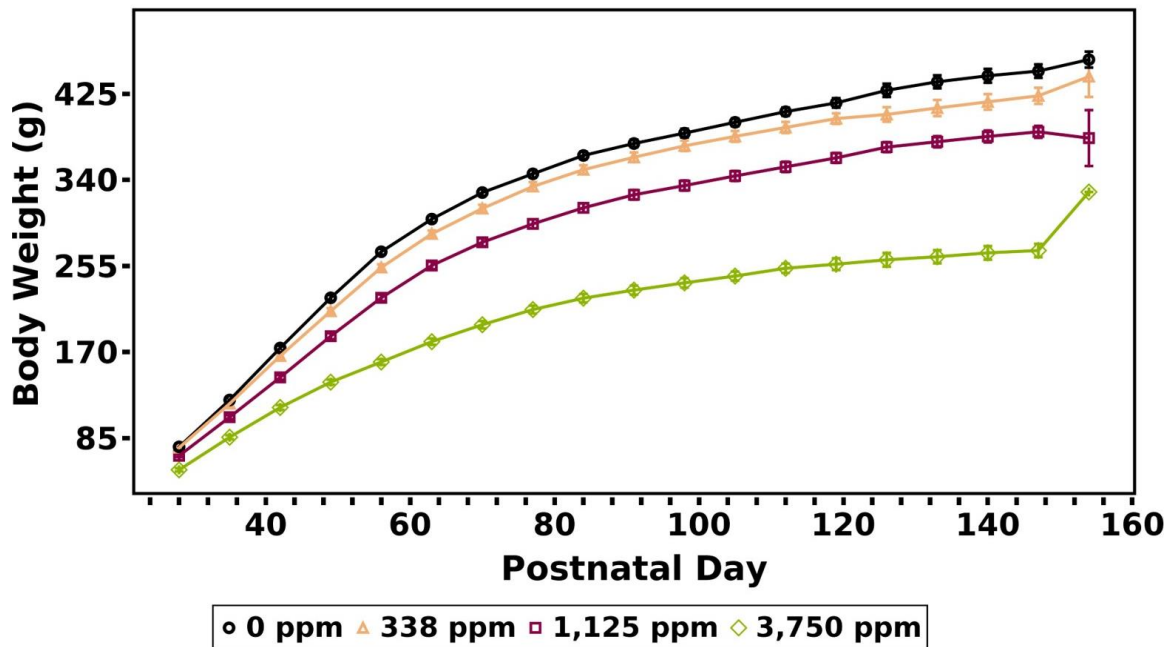


Figure 15. Postweaning Growth Curves for All F₁ Male Rats Exposed to Bisphenol AF in Feed

Information for statistical significance in F₁ male rat weights is provided in Table 15.

Females (Postweaning)

Female F₁ mean body weights between PND 28 and PND 98 were significantly decreased in the 1,125 and 3,750 ppm groups (Table 16; Figure 16). On PND 28, mean body weights for the 1,125 and 3,750 ppm groups were lower by 9% and 26% relative to the control group, respectively, and by PND 98, mean body weights were lower by 15% and 29% relative to the

control group, respectively. Mean body weights of F₁ females in the 338 ppm group were significantly decreased by approximately 6% compared to the control animals on PND 98.

Similar to the F₁ male pups, a significant decrease in absolute feed consumption (g/animal/day) by the F₁ females in the 3,750 ppm group was observed for the PND 28–98 interval (Table 16), but relative feed consumption values (g/kg/day) were significantly increased compared to the control group. This finding is likely due to the reduced size of these pups. Absolute feed consumption by F₁ females in the 338 and 1,125 ppm groups was similar to that of the control group, although a significant increase in relative feed consumption was observed, resulting in a 9% and 16% increase in overall consumption, respectively, compared to the control females.

BPAF intakes for F₁ females, based on feed consumption and dietary concentrations for PND 28–98, were 32, 113, and 411 mg/kg/day at 338, 1,125, and 3,750 ppm, respectively.

Table 16. Summary of Postweaning Mean Body Weights, Body Weight Gains, and Feed and Test Article Consumption of All F₁ Female Rats Exposed to Bisphenol AF in Feed

Postnatal Day ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Body Weight (g)^{b,c}				
28	69.5 ± 1.4** 66 (22)	69.3 ± 1.3 66 (24)	63.5 ± 1.6** 58 (22)	51.5 ± 1.4** 58 (22)
91	238.3 ± 3.3** 53 (22)	223.8 ± 3.6** 55 (24)	202.2 ± 2.3** 53 (22)	169.9 ± 2.2** 48 (22)
98	242.9 ± 3.4** 53 (22)	227.2 ± 3.7** 55 (24)	206.0 ± 2.3** 53 (22)	172.6 ± 2.3** 48 (22)
Body Weight Gain (g)^{b,c}				
28–98	173.3 ± 2.9** 53 (22)	157.8 ± 3.1** 55 (24)	142.6 ± 2.1** 53 (22)	121.2 ± 1.7** 48 (22)
Postweaning Feed Consumption^{d,e}				
28–98 (g/animal/day)	15.6 ± 0.2** (25)	16.3 ± 0.4 (26)	15.6 ± 0.4 (24)	14.5 ± 0.4* (24)
28–98 (g/kg/day)	86.7 ± 1.1** (25)	94.4 ± 1.7** (26)	100.5 ± 2.1** (24)	109.5 ± 1.9** (24)
Chemical Intake (mg/kg/day)^{e,f,g}				
28–98	0.0 ± 0.0 (25)	31.9 ± 0.6 (26)	113.0 ± 2.3 (24)	410.8 ± 7.2 (24)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData are displayed as mean ± standard error (n). Feed consumption values were excluded when excessive spillage was recorded.

^bStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple comparisons.

^cn = the number of pups examined (number of litters).

^dStatistical analysis performed using the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

^en = number of cages.

^fChemical intake calculated as: $([\text{exposure concentration} \times \text{feed consumption}] / [\text{average body weight of day range}])$.

^gNo statistical analysis performed on the chemical intake data.

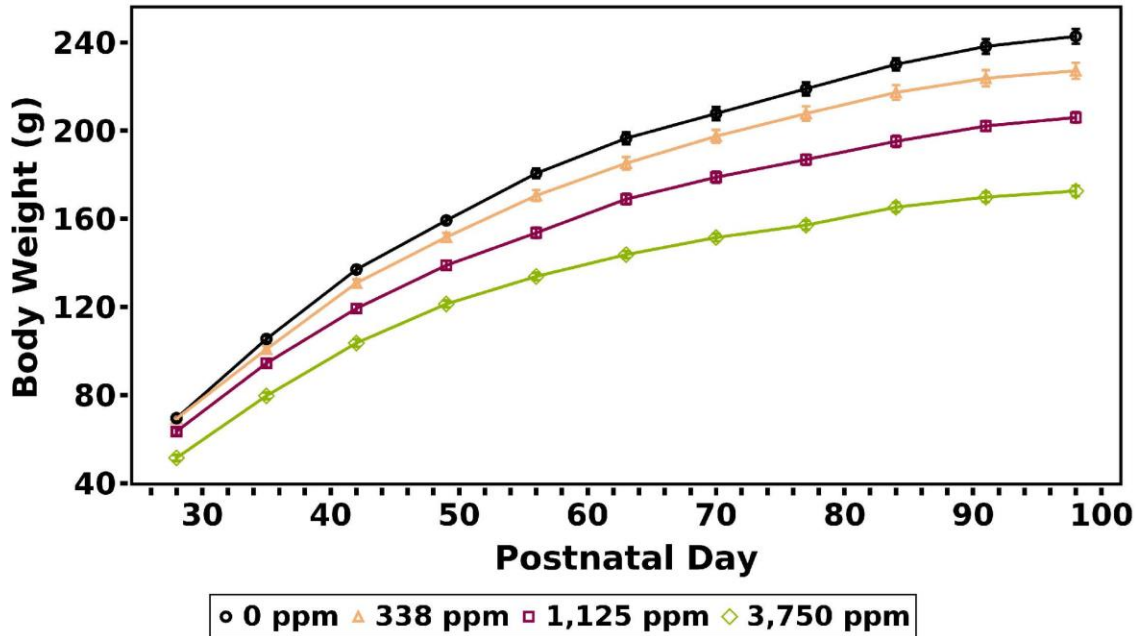


Figure 16. Postweaning Growth Curves for All F₁ Female Rats Exposed to Bisphenol AF in Feed

Information for statistical significance in F₁ female rat weights is provided in Table 16.

Developmental Endpoints

Anogenital Distance

F₁ and F₂ male and female offspring exposed to BPAF did not display any pairwise significant alterations in anogenital distance (AGD) or in PND 1 mean body weight-adjusted AGD; however, a positive trend in body weight-adjusted AGD with exposure concentration was noted for the F₁ females (Table 17).

Table 17. Summary of Anogenital Distance of F₁ and F₂ Male and Female Rats Exposed to Bisphenol AF in Feed

Parameter ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
F₁ Males				
No. Examined ^b	135 (24)	168 (28)	178 (28)	136 (23)
Adjusted AGD (mm) ^{c,d}	2.22 ± 0.03	2.19 ± 0.03	2.20 ± 0.03	2.22 ± 0.04
F₂ Males				
No. Examined	79 (17)	108 (19)	32 (7)	— ^e
Adjusted AGD (mm)	2.12 ± 0.03	2.22 ± 0.03	2.14 ± 0.04	—
F₁ Females				
No. Examined	179 (24)	151 (27)	176 (28)	127 (22)
Adjusted AGD (mm)	1.10 ± 0.02*	1.11 ± 0.02	1.09 ± 0.02	1.17 ± 0.03

Bisphenol AF, NTP DART 08

Parameter ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
F₂ Females				
No. Examined	118 (18)	102 (19)	26 (7)	–
Adjusted AGD (mm)	1.14 ± 0.03	1.10 ± 0.03	1.07 ± 0.02	–

Statistical significance for the vehicle control group indicates a significant trend test.

^aStatistically significant at $p \leq 0.05$.

PND = postnatal day; AGD = anogenital distance.

^aData are displayed as mean ± standard error unless otherwise noted; values are based on litter means, not individual pup values. Animals found dead, cannibalized, or missing (presumed dead) were excluded from analysis.

^bNo. Examined = number of pups examined (number of litters represented).

^cStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

^dAdjusted AGD calculated using the formula: adjusted AGD = raw AGD – (slope*[body weight for that animal – overall body weight mean]), where the slope is the regression slope of AGD versus body weight.

^eNo F₁ females were confirmed pregnant for the 3,750 ppm group in either the prenatal or reproductive performance cohorts.

Areolae/Nipple Retention on PND 13

F₁ and F₂ male offspring exposed to BPAF did not display any signs of areolae/nipple retention (Appendix E).

Testicular Descent

There was no acceleration or delay in day of testicular descent for F₁ males; however, there was one male in the 1,125 ppm group and 11 males out of 7 litters in the 3,750 ppm group that did not attain testicular descent (Table 18; Figure 17). F₂ males exhibited a significant delay in testicular descent of approximately 2 days in the 1,125 ppm group compared to the control group.

Table 18. Summary of Testicular Descent of F₁ and F₂ Male Rats Exposed to Bisphenol AF in Feed

Parameter ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
F₁ Males				
No. Examined ^b	102 (23)	113 (24)	111 (25)	102 (22)
No. Removed ^c	0	0	0	1 (1)
No. Not Attaining ^d	0	0	1 (1)	11 (7)
Mean (Day of Descent) ^{e,f}	18.0 ± 0.2	17.9 ± 0.2	17.7 ± 0.3	18.1 ± 0.5
Proportional Hazards Model, p value ^g	<0.001	0.999	0.999	0.005
F₂ Males				
No. Examined	52 (17)	70 (19)	27 (7)	– ^h
No. Removed	0	0	0	–
No. Not Attaining	1 (1)	0	0	–
Mean (Day of Descent)	15.8 ± 0.4**	16.4 ± 0.3	17.7 ± 0.3*	–
Proportional Hazards Model, p value	0.006	0.476	0.059	–

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData are displayed as mean ± standard error unless otherwise noted; values are based on litter means, not individual pup values.

^bNo. Examined = number of pups examined (number of litters).

^cNo. Removed = number of pups (number of litters) that died or were removed prior to the end of the observation period and did not attain. These animals were excluded from all analyses.

^dNo. Not Attaining = number of pups (number of litters) that survived to the end of the observation period without attaining testicular descent.

^eSummary statistics and mixed model results are presented for animals that attained during the observation period.

^fStatistical analysis performed using mixed models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

^gp values for trend and pairwise comparisons for the proportional hazards analysis were calculated from a Cox proportional hazards model with random effect for litter and a Hommel adjustment for multiple comparisons.

^hNo F₁ females were confirmed pregnant for the 3,750 ppm group in either the prenatal or reproductive performance cohorts.

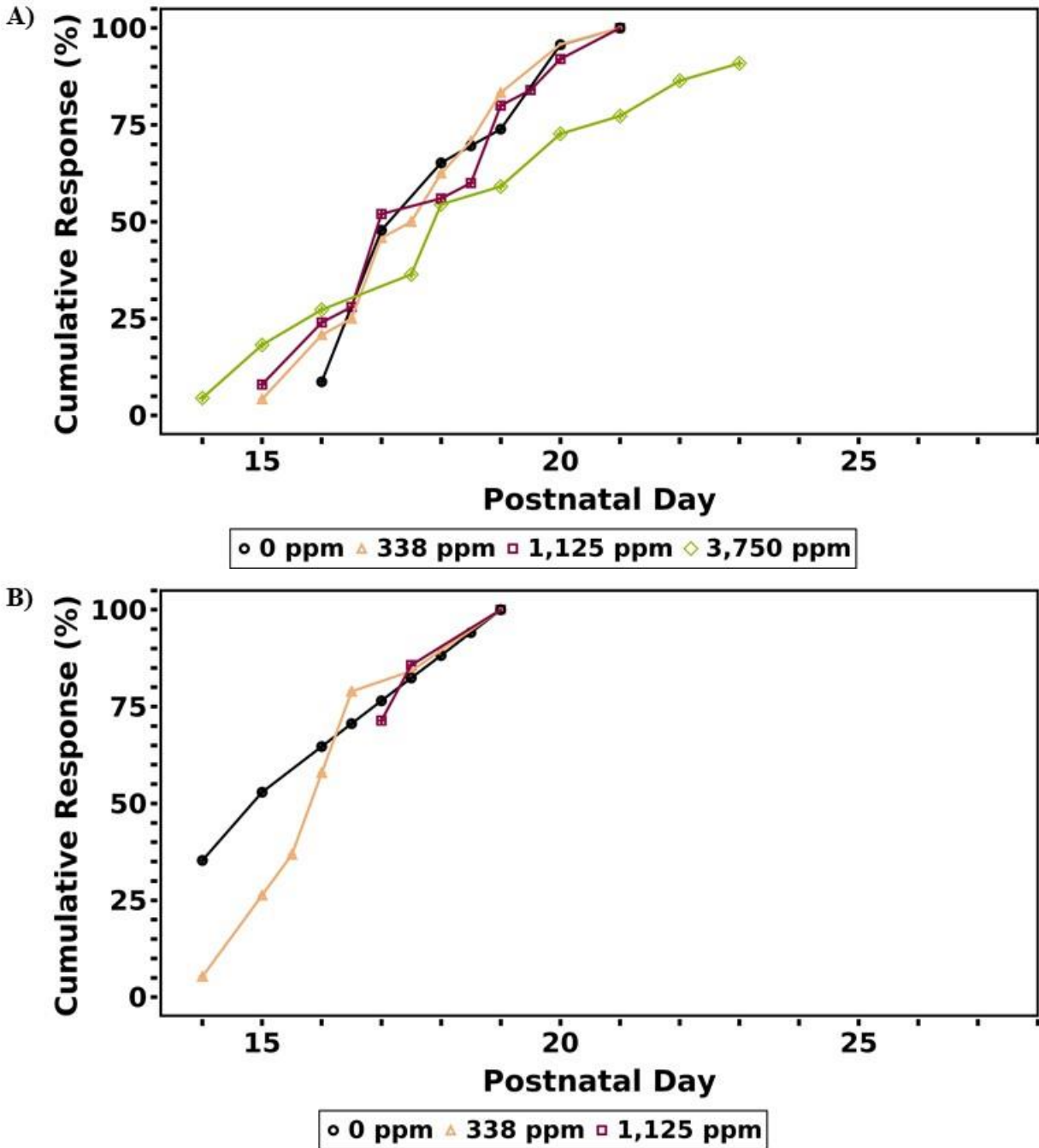


Figure 17. Time to Testicular Descent of F₁ and F₂ Male Offspring Exposed to Bisphenol AF in Feed

Cumulative response curves are shown for (A) F₁ and (B) F₂ males.

Vaginal Opening

All BPAF-exposed females in both the F₁ and F₂ generations exhibited a significant acceleration in litter mean day of vaginal opening (VO) and litter mean day of VO when adjusted for body weight at weaning, relative to the control groups (Table 19). For F₁ and F₂ females, Figure 18 and Figure 19, respectively, show litter and adjusted litter cumulative response (%), or cumulative probability of attainment, plotted against PND for each exposure group. The litter cumulative response curves display an exposure concentration-related shift to the left for unadjusted values. For the F₁ generation, when weaning body weight was used to adjust day of VO attainment, the shift was slightly less pronounced, at approximately 2, 8, and 8 days in the 338, 1,125, and 3,750 ppm groups, respectively. For the F₂ generation, the shift was approximately 3 and 10 days for the 338 and 1,125 ppm groups, respectively (there were no F₂ pups in the 3,750 ppm group).

Table 19. Summary of Vaginal Opening of F₁ and F₂ Female Rats Exposed to Bisphenol AF in Feed

Parameter ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
F₁ Females				
No. Examined ^b	66 (22)	67 (24)	65 (22)	60 (22)
No. Not Attaining ^c	0	0	0	0
Day of VO				
Litter mean ^{d,e}	34.6 ± 0.3**	32.8 ± 0.4*	27.8 ± 0.4**	30.0 ± 0.8**
Adjusted litter mean ^{d,e,f}	35.8 ± 0.3**	33.8 ± 0.3**	27.8 ± 0.3**	27.9 ± 0.7**
Mean Body Weight at Acquisition (g) ^g	103.2 ± 1.7**	90.9 ± 1.6**	63.2 ± 1.4**	60.5 ± 2.6**
Mean Body Weight at Weaning (g) ^g	71.4 ± 1.6**	70.3 ± 1.3	64.9 ± 1.7**	53.0 ± 1.4**
F₂ Females				
No. Examined	78 (18)	77 (19)	20 (7)	— ^h
No. Not Attaining	0	0	0	—
Day of VO				
Litter mean	34.3 ± 0.3**	31.6 ± 0.6**	25.8 ± 0.5**	—
Adjusted litter mean	34.7 ± 0.3**	31.3 ± 0.6**	25.1 ± 0.5**	—
Mean Body Weight at Acquisition (g)	113.8 ± 1.8**	94.3 ± 2.5**	65.9 ± 2.2**	—
Mean Body Weight at Weaning (g)	82.3 ± 1.7*	77.8 ± 1.2	75.2 ± 1.8*	—

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

VO = vaginal opening.

^aData are displayed as mean ± standard error unless otherwise noted; values are based on litter means, not individual pup values.

^bNo. Examined = the number of pups examined (number of litters).

^cNo. Not Attaining = number of pups that survived to the end of the observation period without attaining VO.

^dSummary statistics and mixed model results are presented for animals that attained during the observation period.

^eStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

^fAdjusted based on body weight at weaning. Associated mixed model results reflect inclusion of weaning weight as a covariate.

^gAnalysis of body weight at acquisition and body weight at weaning for both linear trend and pairwise comparisons performed using mixed effects models with litter as a random effect and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

^hNo F₁ females were confirmed pregnant for the 3,750 ppm group in either the prenatal or reproductive performance cohorts.

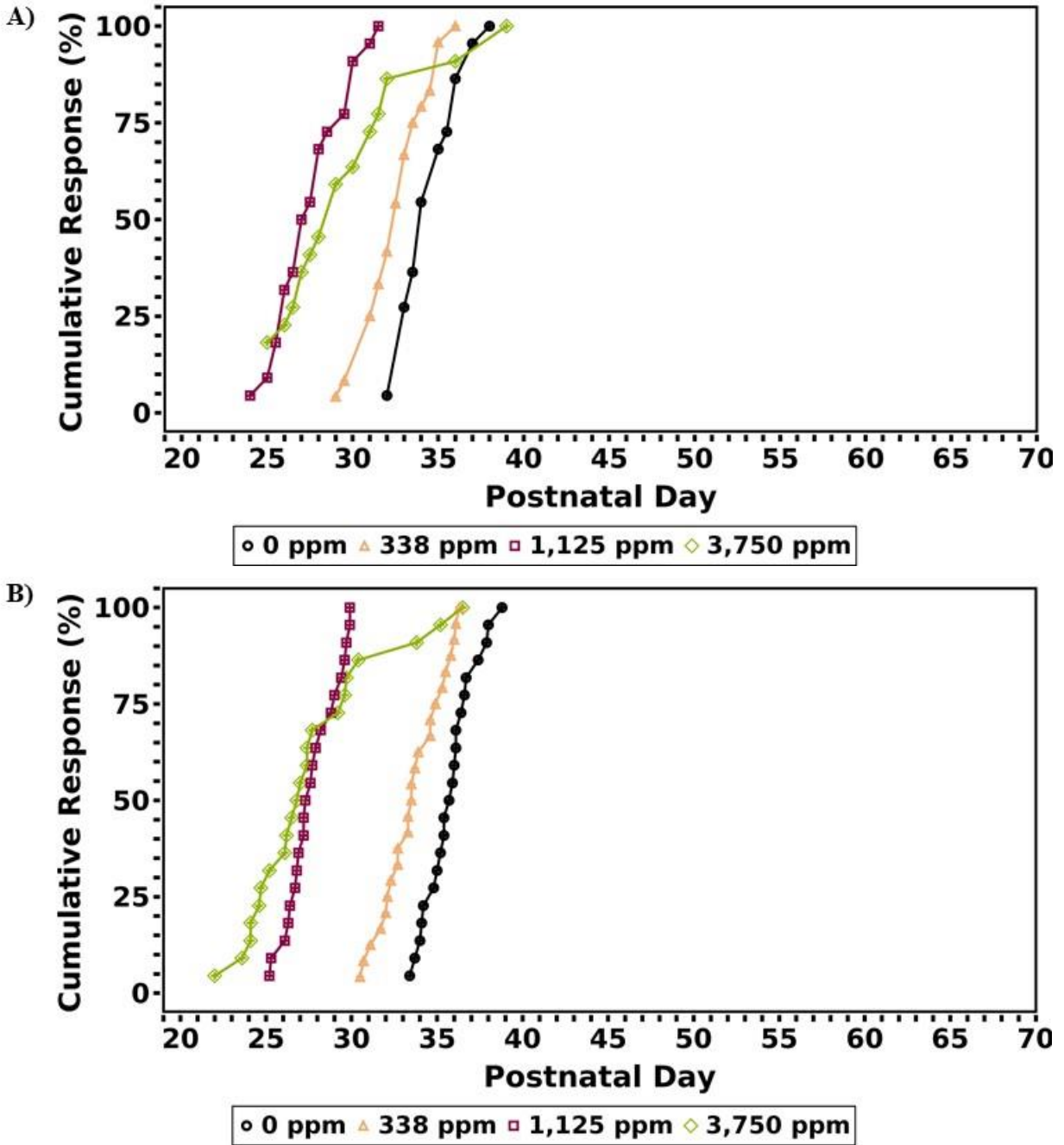


Figure 18. Time to Vaginal Opening of F₁ Female Offspring Exposed to Bisphenol AF in Feed

Cumulative response curves are shown for (A) litter response and (B) litter response adjusted for body weight at weaning.

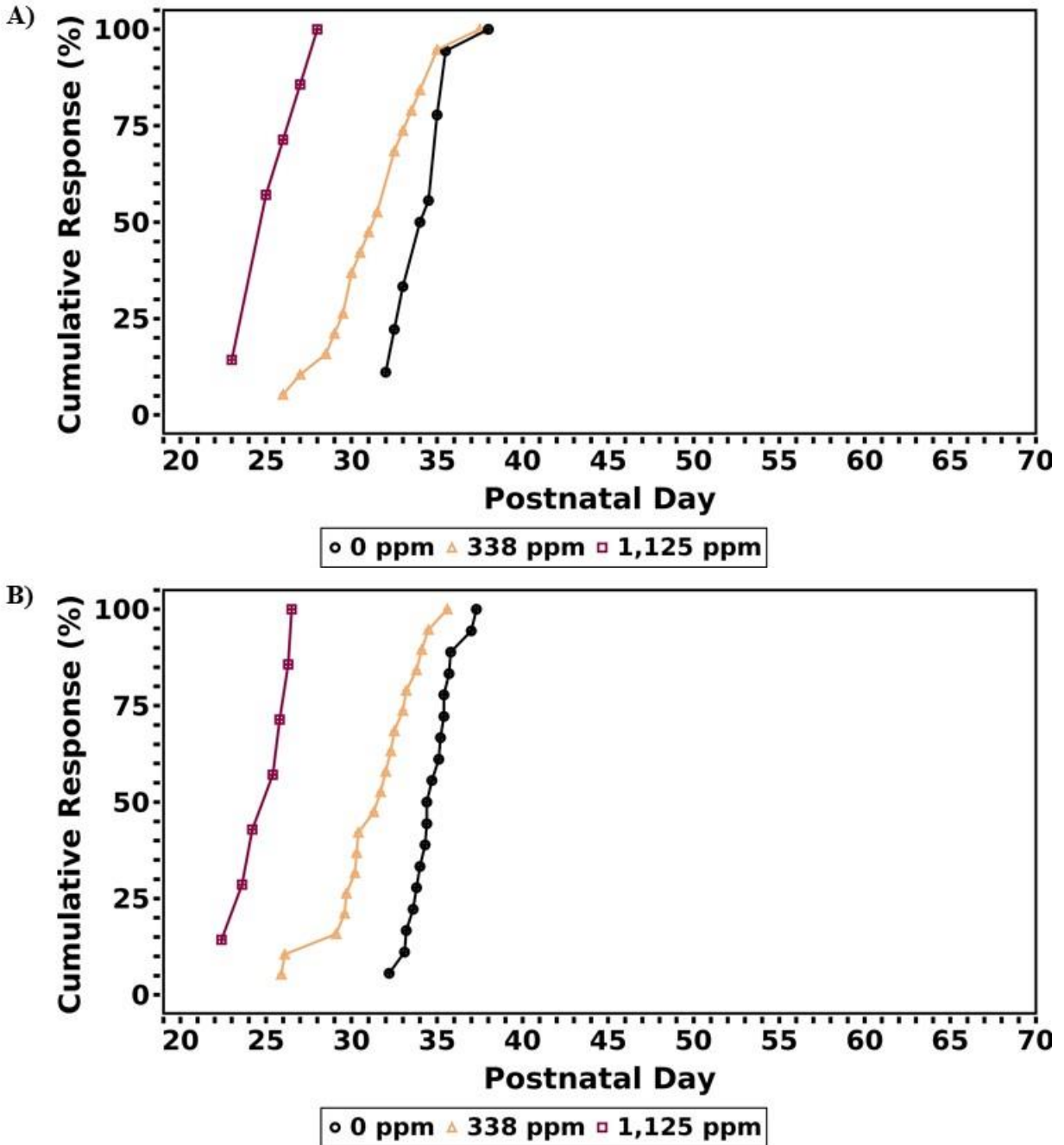


Figure 19. Time to Vaginal Opening of F₂ Female Offspring Exposed to Bisphenol AF in Feed

Cumulative response curves are shown for (A) litter response and (B) litter response adjusted for body weight at weaning.

Balanopreputial Separation

F₁ and F₂ male rats in the 1,125 and 3,750 (F₁ only) ppm groups displayed a significant delay in litter mean day of attaining balanopreputial separation (BPS) and litter mean day of BPS when adjusted for body weight at weaning, relative to the control groups (Table 20). Figure 20 and Figure 21 show litter and adjusted litter cumulative response (%), or cumulative probability of attainment, plotted against PND for each exposure for F₁ and F₂ males, respectively. The litter cumulative response curves for these exposure groups display an exposure concentration-related shift to the right for unadjusted values. When weaning body weight was used to adjust day of BPS attainment, the shift was approximately 4 and 32 days in the 1,125 and 3,750 ppm groups, respectively, for the F₁ generation. The shift was approximately 6 days for the F₂ generation at 1,125 ppm (there were no F₂ pups in the 3,750 ppm group). Ten F₁ males from nine litters in the 3,750 ppm group did not achieve BPS as of PND 98, when checks for this marker stopped.

Table 20. Summary of Balanopreputial Separation of F₁ and F₂ Male Rats Exposed to Bisphenol AF in Feed

Parameter ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
F₁ Males				
No. Examined ^b	54 (22)	55 (24)	53 (22)	50 (20)
No. Not Attaining ^c	0	0	0	10 (9)
Day of BPS				
Litter mean ^{d,e}	45.6 ± 0.3**	45.3 ± 0.4	51.1 ± 0.7**	80.0 ± 2.1**
Adjusted litter mean ^{d,e,f}	46.4 ± 0.2**	46 ± 0.3	50.8 ± 0.7**	78.3 ± 2.1**
Proportional hazards analysis model, p value ^g	<0.001	0.115	<0.001	<0.001
Mean Body Weight at Acquisition (g) ^h	200.4 ± 1.8**	188.0 ± 2.1**	195.1 ± 3.5	217.9 ± 3.6**
Mean Body Weight at Weaning (g) ^h	77.5 ± 1.3**	77.0 ± 1.6	68.6 ± 1.5**	55.4 ± 1.7**
F₂ Males				
No. Examined	52 (17)	70 (19)	27 (7)	— ⁱ
No. Not Attaining	0	0	0	—
Day of BPS				
Litter mean	45.7 ± 0.7**	44.8 ± 0.3	53.3 ± 1.3**	—
Adjusted litter mean	46.5 ± 0.7**	45.0 ± 0.4	52.1 ± 1.1**	—
Mean Body Weight at Acquisition (g)	209.5 ± 4.4	195.1 ± 2.6*	222.1 ± 7.4	—
Mean Body Weight at Weaning (g)	89.4 ± 2.4*	86.8 ± 1.5	79.9 ± 4.2	—

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

BPS = balanopreputial separation.

^aData are displayed as mean ± standard error unless otherwise noted; values are based on litter means, not individual pup values.

^bNo. Examined = the number of pups examined (number of litters).

^cNo. Not Attaining = number of pups (number of litters) that survived to the end of the observation period without attaining BPS.

^dSummary statistics and mixed model results are presented for animals that attained during the observation period.

^eStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

^fAdjusted based on body weight at weaning. Associated mixed model results reflect inclusion of weaning weight as a covariate.

^gp values for trend and pairwise comparisons for the proportional hazards analysis were calculated from a Cox proportional hazards model with exposure concentration and weaning weight as covariates and a random effect for litter and a Hommel adjustment for multiple comparisons.

^hAnalysis of body weight at acquisition and body weight at weaning for both linear trend and pairwise comparisons performed using mixed effects models with litter as a random effect and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

ⁱNo F₁ females were confirmed pregnant for the 3,750 ppm group in either the prenatal or reproductive performance cohorts.

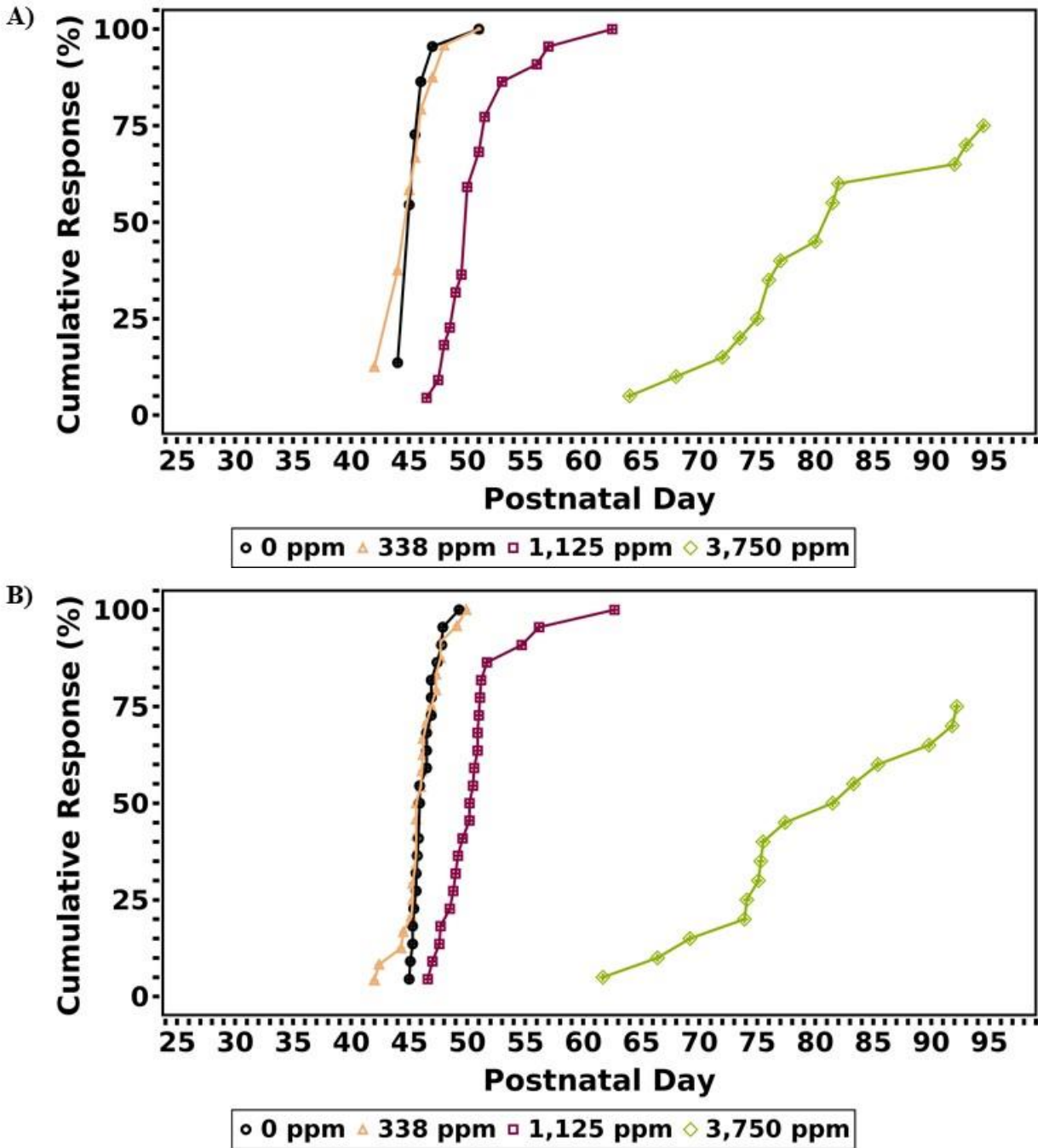


Figure 20. Time to Balanopreputal Separation of F₁ Male Offspring Exposed to Bisphenol AF in Feed

Cumulative response curves are shown for (A) litter response and (B) litter response adjusted for body weight at weaning.

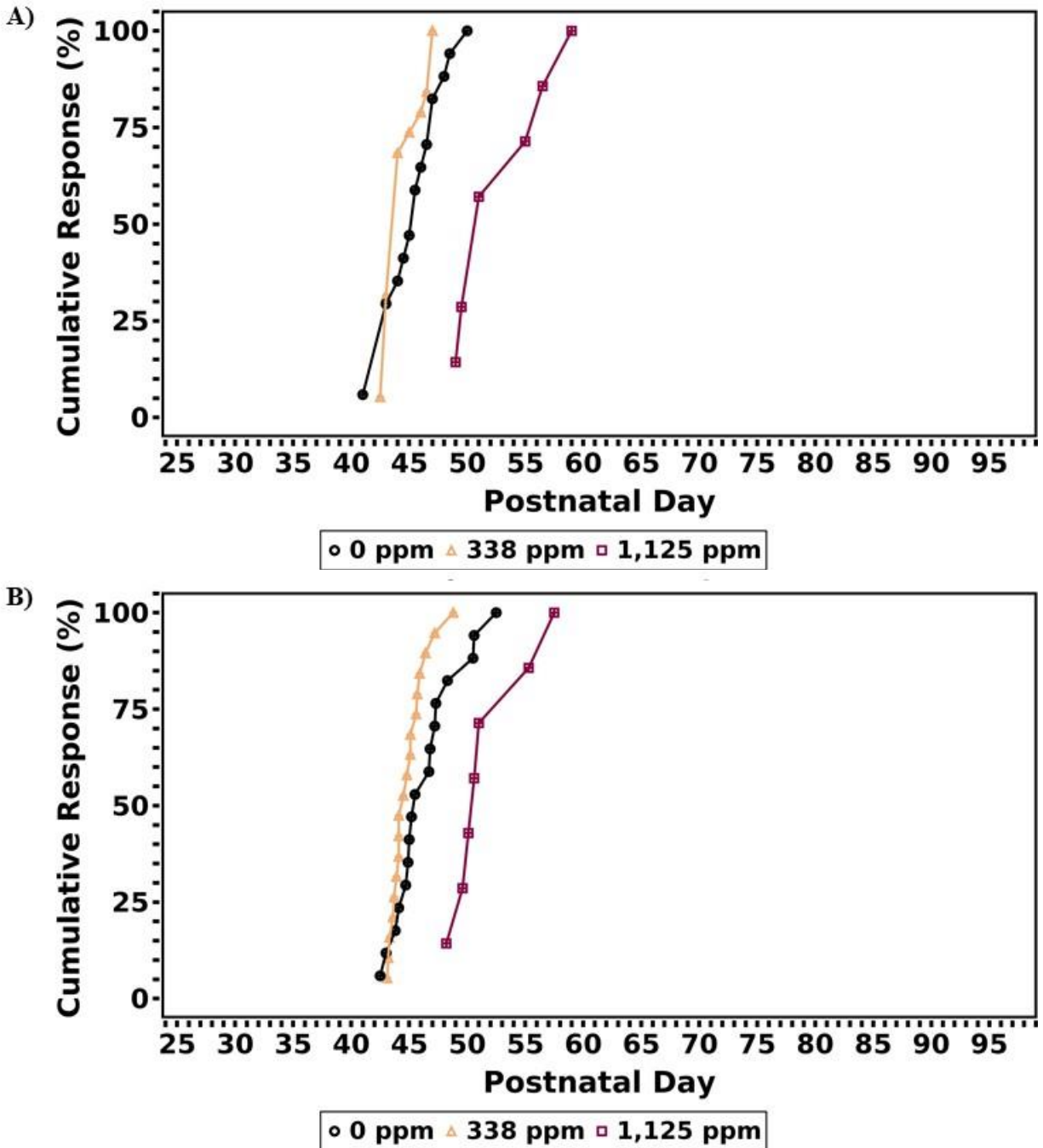


Figure 21. Time to Balanopreputal Separation of F₂ Male Offspring Exposed to Bisphenol AF in Feed

Cumulative response curves are shown for (A) litter response and (B) litter response adjusted for body weight at weaning.

F₁ Cohort Data

Prenatal and Reproductive Performance Cohorts: Mating and Fertility

F₁ male and female rats from the prenatal and reproductive performance cohorts were mated and evaluated for reproductive endpoints, as shown in Figure 22. Viability, clinical observations, vaginal estrous cyclicity, fertility, andrology, mean body weights, and feed consumption results are presented below.

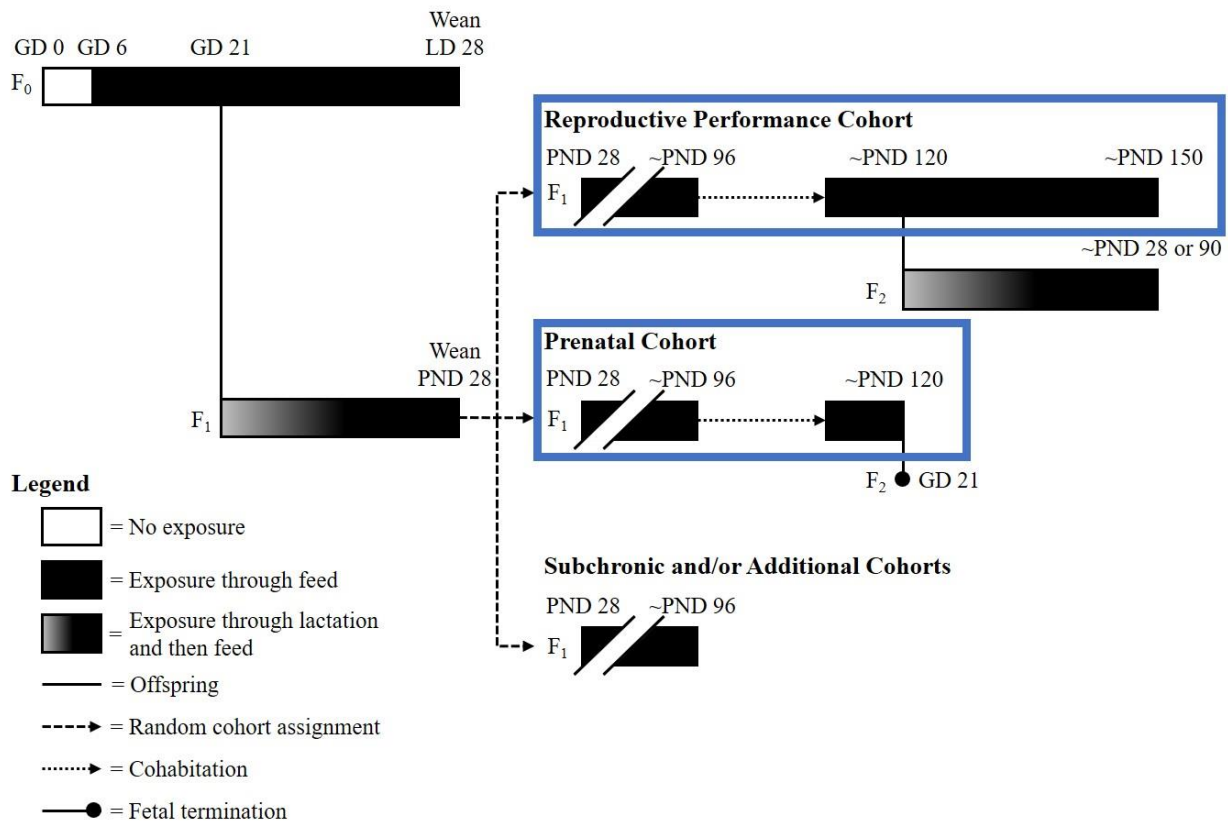


Figure 22. Design of the Modified One-Generation Study – Prenatal and Reproductive Performance Cohorts

GD = gestation day; LD = lactation day; PND = postnatal day.

Viability and Clinical Observations

There were no exposure-related deaths. Clinical observations associated with BPAF exposure were limited to small testis or missing testis in the 3,750 ppm group males and no apparent vaginal opening for one female in the 3,750 ppm group. A second female in the 3,750 ppm group with no vaginal opening was found in the biological sampling cohort (Appendix E). All other clinical observations were singular incidences found across all groups, including the control group.

Selection and Mating

One male and one female rat (1:1) from each litter were allocated to the prenatal and reproductive performance cohorts, avoiding sibling mating. Vaginal lavage samples were

collected for approximately 2 weeks until evidence of mating or until the cohabitation period was completed.

Vaginal Cytology

Estrous cyclicity was assessed in F₁ females allocated to the prenatal, reproductive performance, and subchronic cohorts, and analysis was performed on combined F₁ cohorts. For the F₁ cohorts, estrous cycle length was significantly longer than the control group in the 1,125 ppm group (Table 21). There were no exposure-related changes in number of cycles for the animals that were cycling. In the 3,750 ppm group, 42 out of 47 animals were not cycling and in persistent estrus (Appendix E). Model-based estimates of stage lengths for the 3,750 ppm group were significantly different from the control group for length of estrus (approximately 12 days longer than the control group, $p < 0.01$), proestrus (approximately one-third of a day longer than the control group, $p < 0.01$), and diestrus (approximately 2 days shorter than the control group, $p < 0.01$) (Table 21; Figure 23). For the F₂ cohort, estrous cycle length was significantly longer than the control group in the 338 and 1,125 ppm groups (Table 21). Model-based estimates of stage lengths for the 338 ppm group were significantly different from the control group for length of proestrus (approximately 0.1 days shorter than the control group, $p < 0.01$) and estrus (approximately 0.2 days shorter than the control group, $p < 0.05$) (Table 21; Figure 24). There were no 3,750 ppm F₂ females due to no pregnancies in F₁ females mated at 3,750 ppm.

Table 21. Summary of Estrous Cycle Data and Markov Model Estimates of Estrous Stage Length and 95% Confidence Intervals for All F₁ and F₂ Female Rats Exposed to Bisphenol AF in Feed

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
F₁ Females				
No. of Regular Cycling Females ^a	46 (22)	48 (23)	46 (22)	5 (4)
Estrous Cycle Length (days) ^b	4.97 ± 0.26	5.06 ± 0.18	5.40 ± 0.18*	— ^c
Estrous Stage Length ^{d,e}				
Diestrus	2.7 (2.4, 3.2)	2.8 (2.4, 3.2)	3.3 (3.0, 3.7)	0.5** (0.3, 0.7)
Proestrus	0.4 (0.3, 0.4)	0.3 (0.2, 0.4)	0.4 (0.3, 0.5)	0.7** (0.4, 1.0)
Estrus	1.4 (1.3, 1.5)	1.3 (1.2, 1.4)	1.4 (1.1, 1.7)	13.1** (7.8, 23.5)
Metestrus ^f	0.1	0.1	0.1	0.1
F₂ Females				
No. of Regular Cycling Females	71 (18)	71 (19)	20 (7)	— ^g
Estrous Cycle Length (days)	4.88 ± 0.21	5.11 ± 0.19*	5.22 ± 0.17*	— ^g
Estrous Stage Length				
Diestrus	2.1 (1.9, 2.4)	2.2 (2.0, 2.5)	2.4 (2.0, 2.9)	— ^g
Proestrus	0.2 (0.2, 0.3)	0.1** (0.0, 0.1)	0.1 (0.1, 0.2)	— ^g
Estrus	1.5 (1.4, 1.6)	1.3* (1.2, 1.4)	1.4 (1.1, 1.7)	— ^g
Metestrus	0.2	0.2	0.2	— ^g

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aNo. of Regular Cycling Females = number of animals cycling (number of litters).

^bEstrous cycle length data are presented as mean ± standard error. Animals not cycling were excluded from the cycle length calculation. Pairwise tests performed using the Datta-Satten modified Wilcoxon test with a Hommel adjustment for multiple comparisons.

^cCycle length and number of cycles were not calculated for the 3,750 ppm group due to the large number of animals that were not cycling.

^dEstrous stage length data are presented as days (95% confidence interval).

^ePairwise tests are performed using a permutation null hypothesis testing method and have been adjusted for multiple comparisons using a Hommel correction within each stage.

^fDue to a very low number of observations of metestrus, stage lengths were estimated using a profile likelihood approach. As a result, confidence intervals are not available for the metestrus stage length estimate.

^gNo F₁ females were confirmed pregnant for the 3,750 ppm group in either the prenatal or reproductive performance cohorts.

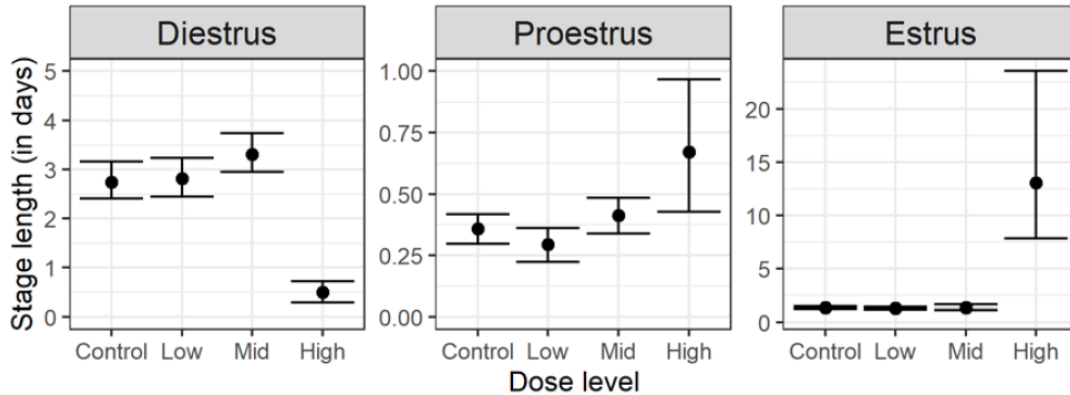


Figure 23. Markov Model Estimates of Stage Lengths and 95% Confidence Intervals for F₁ Female Rats Exposed to Bisphenol AF in Feed

Dots = estimated stage lengths; bars = 95% confidence intervals; low = 338 ppm; mid = 1,250 ppm; high = 3,750 ppm. Metestrus estimates are not shown here due to very low numbers of observations of this stage. Y-axis scales differ for each stage.

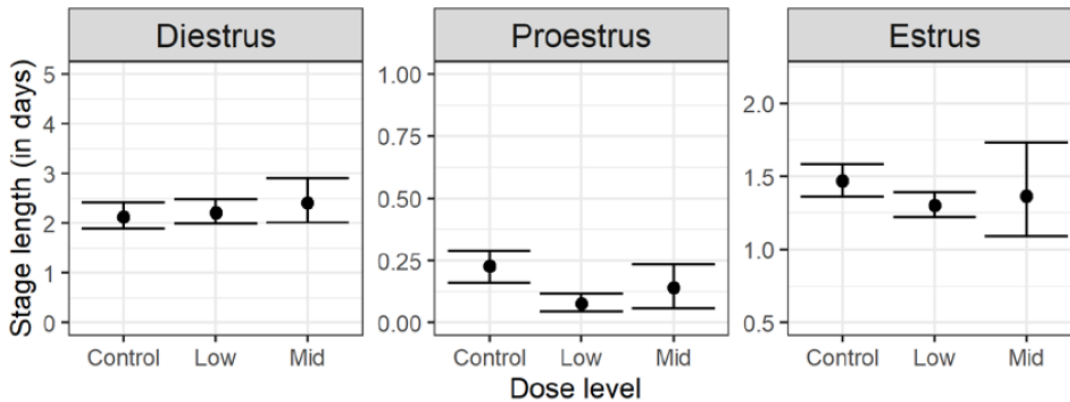


Figure 24. Markov Model Estimates of Stage Lengths and 95% Confidence Intervals for F₂ Female Rats Exposed to Bisphenol AF in Feed

Dots = estimated stage lengths; bars = 95% confidence intervals; low = 338 ppm; mid = 1,250 ppm. Metestrus estimates are not shown here due to very low numbers of observations of this stage. There were no results for the high-exposure concentration (3,750 ppm) group due to a lack of pregnant F₁ females in that group. Y-axis scales differ for each stage.

Fertility

There were no pregnant F₁ females in the 3,750 ppm group for either the prenatal or reproductive performance cohorts, indicating that F₁ male and/or female fertility was affected by BPAF exposure in the 3,750 ppm group. For both the prenatal and reproductive performance cohorts, there was a negative trend with exposure concentration for the percentage of paired females that mated, with significant decreases in the 3,750 ppm groups. A significant decrease was also noted in the reproductive performance cohort at 1,125 ppm but not in the prenatal cohort; this result is possibly due to the differences in control group values. For the reproductive performance cohort, there was a negative trend with exposure concentration for the percentage of mated females that became pregnant and the percentage of mated females that littered; however, no trend was observed for percentage of mated females that became pregnant in the prenatal cohort (Table 22).

Table 22. Summary of Mating and Fertility Performance of F₁ Male and Female Rats Exposed to Bisphenol AF in Feed

Parameter	0 ppm		338 ppm		1,125 ppm		3,750 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC
No. Mating Pairs	22	21	23	21	21	22	19	19
No. Mated	22	17	23	20	16	19	1	0
No. Females Pregnant	18	17	22	20	12	18	0	0
Percent of Mated Females/Paired ^{a,b}	100.0**	81.0**	100.0	95.2	76.2*	86.4	5.3**	0.0**
Percent of Pregnant Females/Mated ^{a,b}	81.8*	100.0	95.7	100.0	75.0	94.7	0.0	– ^c
Percent of Littered Females/Mated ^{a,b}	81.8**	– ^d	87.0	–	56.3	–	0.0	–
Precoital Interval ^{e,f}	6.4 ± 0.7* (20)	4.1 ± 0.9 (16)	5.3 ± 0.9 (20)	4.0 ± 0.7 (20)	4.1 ± 1.2 (13)	3.9 ± 0.7 (16)	1.0 ± 0.0 (1)	(0)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

RPC = reproductive performance cohort; PC = prenatal cohort.

^aStatistical analysis performed using the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.

^bAnimals removed from the study between mating and littering were excluded from calculations of % littered females.

^cPercent of pregnant females/mated was not calculated for the 3,750 ppm prenatal females because there were no mated females.

^dF₁ prenatal females were sectioned prior to littering, so endpoints involving number of females littering were not calculated.

^eStatistical analysis performed using the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

^fPrecoital interval in days is calculated for sperm-positive females; data are displayed as mean ± standard error (n).

F₁ Reproductive Performance Cohort Andrology

There were no BPAF-related effects on motile sperm or progressively motile sperm, but there was a significant increase of 23% above the control group in testis spermatid head concentration (per gram testis) in the 3,750 ppm group (Table 23). Males in the 1,125 and 3,750 ppm groups displayed significant exposure concentration-dependent decreases in cauda epididymal sperm counts (approximately 19% and 58% less than the control group, respectively), epididymis weights (approximately 11% and 40% less than the control group, respectively), and testis weights (approximately 8% and 28% less than the control group, respectively) (Table 23). These findings were associated with histopathological changes in both the testis and epididymis (Appendix E).

Table 23. Summary of Reproductive System Parameters of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed

Parameter ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. Examined on PND 152–154 ^b	21 (21)	23 (23)	21 (21)	20 (20)
Weights (g) ^{c,d}				
Left cauda epididymis	0.262 ± 0.004**	0.249 ± 0.004	0.222 ± 0.006**	0.130 ± 0.007**
Left epididymis	0.673 ± 0.009**	0.648 ± 0.010	0.602 ± 0.013**	0.405 ± 0.020**
Left testis	2.039 ± 0.026**	1.965 ± 0.028	1.876 ± 0.047**	1.469 ± 0.057**

Bisphenol AF, NTP DART 08

Parameter ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Spermatid Measurements ^c				
Spermatid heads (10 ⁶ /g testis)	120.9 ± 3.9**	128.5 ± 3.5	128.0 ± 3.9	148.8 ± 6.3**
Spermatid heads (10 ⁶ /testis)	246.4 ± 8.5*	252.6 ± 8.1	237.9 ± 7.2	216.4 ± 9.6
Epididymal Spermatozoal Measurements ^c				
Sperm motility (%)	65.0 ± 3.6	64.4 ± 2.9	57.5 ± 3.5	63.4 ± 3.8
Sperm progressive motility (%)	53.0 ± 3.1	50.3 ± 2.1	45.7 ± 3.2	50.7 ± 3.3
Sperm (10 ⁶ /g cauda epididymis)	843.4 ± 27.3**	835.2 ± 26.3	796.9 ± 38.3	704.1 ± 27.1**
Cauda epididymis sperm count (10 ⁶ /cauda epididymis)	221.5 ± 8.3**	207.5 ± 6.5	179.9 ± 11.4**	94.0 ± 8.1**

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

PND = postnatal day.

^aData are presented as mean ± standard error.

^bNo. Examined on PND 152–154 = the number of pups examined (number of litters). Spermatid head concentration for one animal in the 1,125 ppm group was excluded as an outlier.

^cStatistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^dIf there was a lesion in the left organ, the contralateral tissue was taken.

^eStatistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

F₁ Gestation Body Weights

As previously noted, F₁ female rats exposed to BPAF displayed both lower preweaning and postweaning mean body weights. Consequently, the F₁ female mean body weights of the 338 and 1,125 ppm groups in both the prenatal and reproductive performance cohorts at the time of cohabitation were lower relative to control females and were 4%–7% and 15%–17% lower at GD 0, respectively (no pregnancies occurred in the 3,750 ppm group for either the prenatal or reproductive performance cohorts) (Figure 25, Figure 26). Both cohorts exposed to 338 or 1,125 ppm displayed significantly decreased mean body weight gains throughout the gestational period (approximately 15%–16% and 40%–47%, respectively) relative to their respective control groups (Table 24) and were 9%–10% and 25%–29% lower, respectively, on GD 21. This difference in mean body weight gain during pregnancy for the 1,125 ppm group might be the result of a significant decrease in litter size of approximately five fewer fetuses/pups in this group than in the control group (Appendix E); a decrease in litter size was not observed in the 338 ppm group.

F₁ Gestation Feed Consumption

Absolute (g/animal/day) feed consumption over the GD 0–21 interval was lower in the 338 ppm group (approximately 5% below the control group for both the prenatal and reproductive performance cohorts) and significantly decreased in the 1,125 ppm group (13% and 14% below the control group for the prenatal and reproductive performance cohorts, respectively). Relative feed consumption (g/kg/day) over the GD 0–21 interval was similar to the control group for the 338 ppm group in both cohorts. In the 1,125 ppm group, relative feed consumption was significantly increased and higher in the prenatal and reproductive performance cohorts, respectively, indicating that the lower feed consumption values were relative to the body weight of the animals during gestation (Table 25). BPAF intakes for F₁ females in both cohorts during gestation, based on feed consumption and dietary concentrations for GD 0–21, were approximately 26 and 92 mg/kg/day at 338 and 1,125 ppm, respectively (Table 25), slightly higher than the exposure during the F₀ gestation (24 and 81 mg/kg/day, respectively).

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Table 24. Summary of Gestation Mean Body Weights and Body Weight Gains for F₁ Female Rats Exposed to Bisphenol AF in Feed^{a,b}

Parameter	0 ppm		338 ppm		1,125 ppm		3,750 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC
Gestation Day								
0	249.0 ± 4.5** (16)	246.7 ± 3.7** (16)	239.2 ± 5.1 (19)	229.4 ± 4.1** (20)	210.7 ± 4.9** (9)	203.9 ± 4.8** (15)	– ^c	–
21	407.0 ± 7.5** (16)	415.9 ± 4.9** (16)	372.0 ± 7.6** (19)	373.3 ± 6.6** (20)	305.9 ± 13.3** (9)	294.4 ± 9.2** (15)	–	–
Gestation Day Interval								
0–21	158.0 ± 6.1** (16)	169.2 ± 2.7** (16)	132.8 ± 6.8* (19)	143.9 ± 4.4** (20)	95.3 ± 10.7** (9)	90.4 ± 9.1** (15)	–	–
0–3	18.9 ± 1.5** (16)	16.4 ± 1.4* (16)	14.4 ± 1.0* (19)	13.4 ± 0.7 (20)	11.9 ± 1.6** (9)	13.4 ± 1.0 (15)	–	–
3–6	11.6 ± 1.0** (16)	12.2 ± 1.0** (16)	9.2 ± 0.9* (19)	8.9 ± 0.9* (20)	6.3 ± 0.6** (9)	7.1 ± 1.2** (15)	–	–
6–9	9.7 ± 0.7 (16)	11.1 ± 1.0* (16)	9.5 ± 1.1 (19)	9.1 ± 0.9 (20)	6.9 ± 1.4 (9)	6.8 ± 1.1* (15)	–	–
9–12	11.5 ± 0.9* (16)	13.5 ± 0.9** (16)	9.4 ± 0.9 (19)	12.2 ± 0.6 (20)	6.9 ± 1.6* (9)	6.9 ± 1.0** (15)	–	–
12–15	19.8 ± 1.0** (16)	20.8 ± 0.9** (16)	17.0 ± 1.4 (19)	16.5 ± 1.0* (20)	10.9 ± 2.0** (9)	10.8 ± 1.9** (15)	–	–
15–18	40.4 ± 2.2* (16)	45.9 ± 1.5** (16)	35.0 ± 2.9 (19)	41.0 ± 1.9 (20)	24.4 ± 5.1** (9)	19.4 ± 3.4** (15)	–	–
18–21	46.0 ± 2.7** (16)	49.4 ± 1.8** (16)	38.3 ± 3.3 (19)	42.7 ± 1.5* (20)	27.9 ± 2.8** (9)	26.0 ± 3.1** (15)	–	–

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

RPC = reproductive performance cohort; PC = prenatal cohort.

^aData are displayed as mean ± standard error (n), where n = number of animals. Body weight data are reported in grams.

^bStatistical analysis performed using the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^cNo females were confirmed pregnant for the 3,750 ppm group.

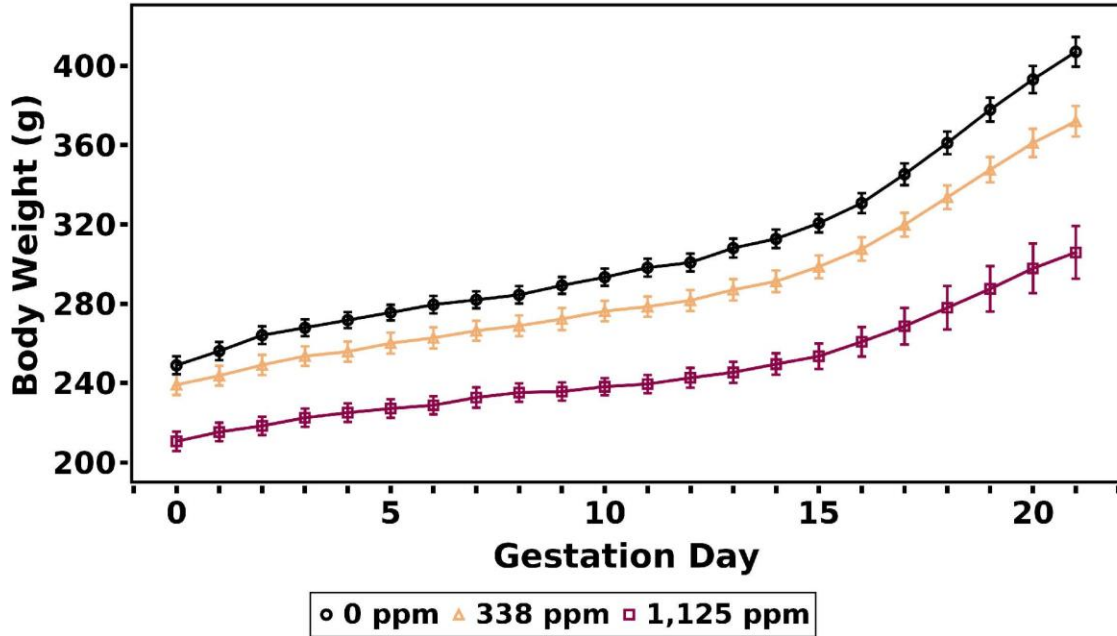


Figure 25. Gestation Growth Curves for F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed

Information for statistical significance in F₁ female rat weights is provided in Table 24.

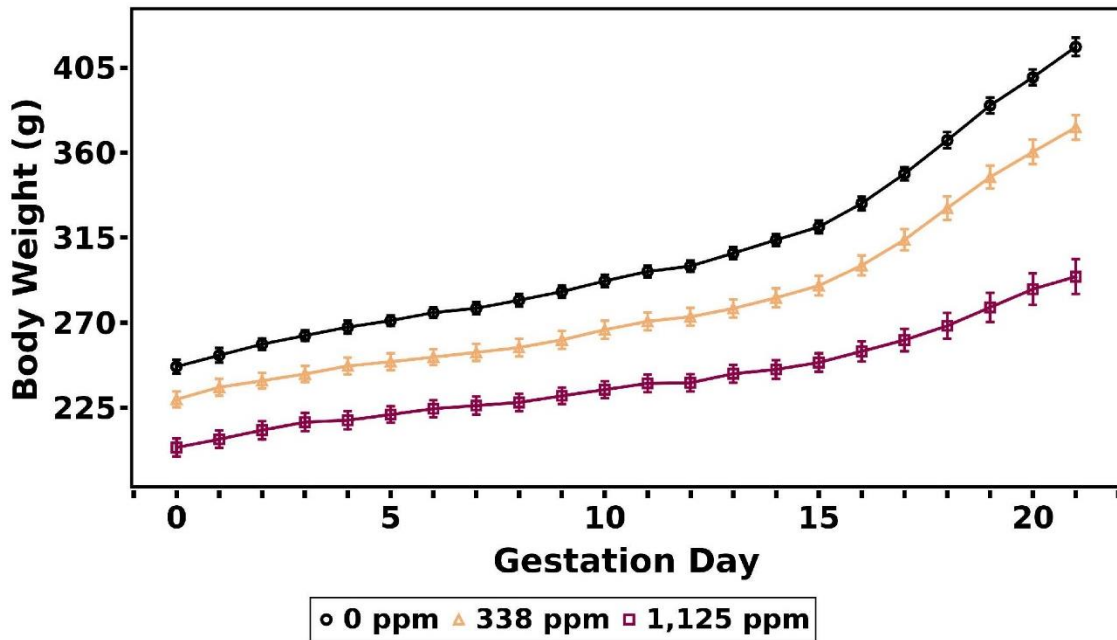


Figure 26. Gestation Growth Curves for F₁ Female Rats in the Prenatal Cohort Exposed to Bisphenol AF in Feed

Information for statistical significance in F₁ female rat weights is provided in Table 24.

Bisphenol AF, NTP DART 08

Table 25. Summary of Gestation Feed and Test Article Consumption for F₁ Female Rats Exposed to Bisphenol AF in Feed^{a,b}

GD Interval	0 ppm		338 ppm		1,125 ppm		3,750 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC
Feed Consumption (g/animal/day)^{c,d}								
0–21	23.5 ± 0.4** (16)	22.7 ± 0.3** (16)	22.3 ± 0.7 (19)	21.6 ± 0.5 (20)	20.1 ± 1.0** (9)	19.7 ± 0.5** (15)	– ^e	–
0–3	21.9 ± 0.6 (16)	19.7 ± 0.3 (16)	21.4 ± 1.0 (18)	20.3 ± 0.9 (15)	20.7 ± 2.0 (7)	22.4 ± 1.4 (10)	–	–
3–6	21.7 ± 0.4** (15)	20.8 ± 0.4** (16)	20.4 ± 0.7 (19)	18.4 ± 0.5** (20)	17.2 ± 0.4** (8)	16.4 ± 0.5** (14)	–	–
6–9	22.3 ± 0.4 (16)	21.2 ± 0.3 (16)	22.8 ± 1.3 (17)	21.9 ± 1.1 (17)	22.5 ± 1.7 (8)	21.5 ± 1.8 (10)	–	–
9–12	21.3 ± 0.4** (16)	20.8 ± 0.4** (16)	20.1 ± 0.6 (18)	19.0 ± 0.4** (19)	16.0 ± 0.4** (9)	16.0 ± 0.6** (15)	–	–
12–15	24.0 ± 0.6 (16)	23.2 ± 0.5 (16)	22.8 ± 0.8 (19)	22.8 ± 1.1 (19)	25.2 ± 2.8 (8)	24.6 ± 1.2 (13)	–	–
15–18	25.2 ± 0.5** (16)	25.8 ± 0.5** (16)	23.6 ± 0.5* (18)	23.3 ± 0.6** (20)	19.0 ± 0.9** (9)	18.7 ± 0.7** (15)	–	–
18–21	27.7 ± 0.6** (15)	27.0 ± 0.6** (16)	24.7 ± 0.9** (19)	24.4 ± 0.8** (20)	21.9 ± 0.9** (7)	22.8 ± 1.0** (9)	–	–
Feed Consumption (g/kg/day)^{c,d}								
0–21	76.4 ± 1.2 (16)	73.8 ± 0.8* (16)	77.6 ± 1.7 (19)	77.3 ± 1.7 (20)	81.4 ± 3.3 (9)	82.4 ± 2.9* (15)	–	–
0–3	84.7 ± 2.5 (16)	77.4 ± 1.6** (16)	87.1 ± 3.5 (18)	86.1 ± 4.1 (15)	94.9 ± 7.4 (7)	106.1 ± 7.2** (10)	–	–
3–6	79.9 ± 1.9 (15)	77.2 ± 1.5 (16)	79.0 ± 2.1 (19)	74.3 ± 1.5 (20)	75.9 ± 1.5 (8)	75.2 ± 2.6 (14)	–	–
6–9	78.8 ± 1.3 (16)	75.8 ± 1.1 (16)	85.4 ± 4.1 (17)	84.9 ± 4.1 (17)	96.7 ± 8.0 (8)	94.9 ± 9.1 (10)	–	–
9–12	72.3 ± 1.2 (16)	70.9 ± 1.1 (16)	72.3 ± 1.4 (18)	71.1 ± 1.2 (19)	67.1 ± 1.7 (9)	68.0 ± 2.1 (15)	–	–
12–15	77.2 ± 1.6 (16)	74.9 ± 1.3** (16)	78.6 ± 2.1 (19)	81.7 ± 4.0 (19)	101.0 ± 10.7* (8)	102.5 ± 5.8** (13)	–	–
15–18	74.4 ± 1.0 (16)	75.5 ± 1.3 (16)	75.4 ± 1.2 (18)	75.4 ± 1.4 (20)	71.6 ± 2.6 (9)	72.2 ± 1.9 (15)	–	–
18–21	72.6 ± 1.3 (15)	69.0 ± 1.3 (16)	70.1 ± 2.4 (19)	69.4 ± 2.3 (20)	76.2 ± 4.4 (7)	76.6 ± 4.6 (9)	–	–
Chemical Intake (mg/kg/day)^{f,g}								
0–21	0.0 ± 0.0 (16)	0.0 ± 0.0 (16)	26.2 ± 0.6 (19)	26.1 ± 0.6 (20)	91.6 ± 3.8 (9)	92.7 ± 3.3 (15)	–	–

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

GD = gestation day; RPC = reproductive performance cohort; PC = prenatal cohort.

^aData are displayed as mean ± standard error (n), where n = number of animals. Consumption is not reported for the nonpregnant animals during gestation.

^bFor each dam, calculation of consumption values for the GD 0–21 interval was performed using all valid data for the animal, even if data were unavailable for some of the subintervals.

^cChanges in n are the result of excluded feed consumption values due to excessive spillage. Additional animal feed consumption values removed as outliers include: GD 3–6 (one RPC female in the 1,125 ppm group) and GD 9–12 (one PC female in the 338 ppm group).

^dStatistical analysis performed using the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^eNo females were confirmed pregnant for the 3,750 ppm group.

^fChemical intake calculated as: $([\text{exposure concentration} \times \text{feed consumption}]/[\text{average body weight of day range}])$.

^gNo statistical analysis performed on the chemical intake data.

Prenatal Cohort Findings

F₁ rats and F₂ fetuses from the prenatal cohort were evaluated for maternal reproductive performance and fetal findings, respectively, as shown in Figure 27.

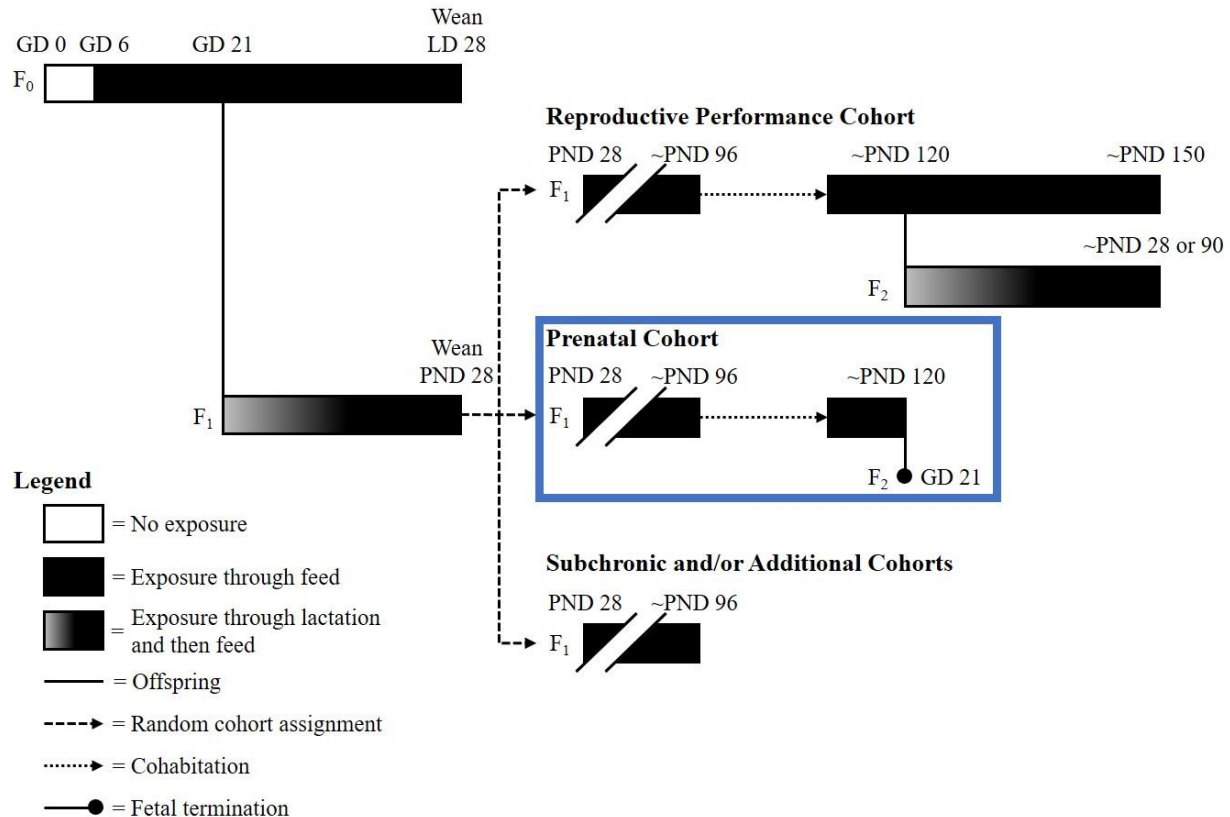


Figure 27. Design of the Modified One-Generation Study – Prenatal Cohort

GD = gestation day; LD = lactation day; PND = postnatal day.

Maternal Reproductive Performance and Uterine Data

In the prenatal cohort, females were between 123 and 137 days of age at the time of necropsy. Pregnant females exposed to 338 or 1,125 ppm BPAF displayed lower gravid uterine weights (13% and 56%, respectively, significant only at 1,125 ppm), and the number of uterine implantations significantly decreased in both exposed groups (there were no pregnant females in the 3,750 ppm group) (Table 26). A significant increase in pre- and postimplantation loss and fewer live fetuses (approximately seven fewer per litter) were observed in the 1,125 ppm group. These findings correlated with significant decreases in the mean number of corpora lutea (approximately four fewer per litter at 1,125 ppm) relative to the control group and are consistent with the significant decreases in live litter size and mean live fetal weights (significantly decreased by 25% compared to the control animals) (Table 26). Dams exposed to BPAF did not display any significant changes in fetal sex ratio.

Table 26. Summary of Uterine Content Data for F₁ Females in the Prenatal Cohort Exposed to Bisphenol AF in Feed

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Pregnancy Summary^a				
Paired Females	21	21	22	19
Mated Females	17**	20	19	0**
Pregnant Females ^b	17	20	18	0
Pregnant Females Examined on GD 21	16	20	15	0
Preimplantation Loss^{c,d}				
Mean No. of Corpora Lutea/Female	15.82 ± 0.44** (17)	14.20 ± 0.47** (20)	11.89 ± 0.52** (18)	— ^e
Implantations/Female	15.00 ± 0.37** (16)	13.85 ± 0.39* (20)	8.73 ± 0.69** (15)	—
Preimplantation Loss (%)	5.40 ± 2.06** (16)	3.77 ± 1.47 (20)	24.44 ± 4.97** (15)	—
Intrauterine Deaths^d				
Postimplantation Loss (%) ^c	2.26 ± 1.52** (16)	4.51 ± 2.26 (20)	25.87 ± 8.78* (15)	—
Total Resorptions per Litter ^c	0.38 ± 0.26* (16)	0.60 ± 0.31 (20)	1.87 ± 0.62* (15)	—
Early Resorptions per Litter ^c	0.38 ± 0.26* (16)	0.60 ± 0.31 (20)	1.87 ± 0.62* (15)	—
Late Resorptions per Litter ^c	0.00 ± 0.00 (16)	0.00 ± 0.00 (20)	0.00 ± 0.00 (15)	—
Dead Fetuses per Litter ^c	0.00 ± 0.00 (16)	0.00 ± 0.00 (20)	0.07 ± 0.07 (15)	—
No. of Early Resorptions	6	12	28	—
No. of Late Resorptions	0	0	0	—
No. of Whole Litter Resorptions ^a	0	0	1	—
No. of Dead Fetuses	0	0	1	—
Live Fetuses^d				
No. of Live Fetuses	234	265	102	—
Live Fetuses per Litter ^c	14.63 ± 0.34 (16)	13.25 ± 0.52 (20)	7.29 ± 1.06** (14)	—
Live Male Fetuses per Litter ^c	7.81 ± 0.44 (16)	7.35 ± 0.47 (20)	3.92 ± 0.73** (13)	—
Live Female Fetuses per Litter ^c	6.81 ± 0.21 (16)	5.90 ± 0.34 (20)	3.64 ± 0.61** (14)	—
Live Male Fetuses per Litter (%) ^c	52.95 ± 1.94 (16)	54.93 ± 2.62 (20)	44.88 ± 6.42 (14)	—
Fetal Weight (g)^{c,f,g}				
Fetal Weight per Litter	5.09 ± 0.07** (16)	4.98 ± 0.06 (19)	3.81 ± 0.35** (14)	—
Male Fetal Weight per Litter	5.20 ± 0.08** (16)	5.09 ± 0.07 (19)	3.96 ± 0.38** (13)	—
Female Fetal Weight per Litter	4.96 ± 0.06** (16)	4.83 ± 0.06 (19)	3.80 ± 0.34** (14)	—
Gravid Uterine Weight (g)^{c,f}				
Gravid Uterine Weight	104.01 ± 2.97** (16)	90.93 ± 3.70 (20)	45.73 ± 8.01** (15)	—
Terminal Body Weight	415.7 ± 4.9** (16)	373.8 ± 6.5** (20)	295.3 ± 9.1** (15)	—
Adjusted Body Weight ^h	311.74 ± 3.31** (16)	282.82 ± 4.41** (20)	249.53 ± 5.28** (15)	—

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

GD = gestation day.

^aStatistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.

^bIncludes animals that had any evidence of pregnancy but were removed from the study before GD 21.

^cData are reported per litter as mean ± standard error (n) and do not include nonmated, nonpregnant, or unexamined animals or those that did not survive to the end of the study. One litter in the 338 ppm group was excluded from fetal weight analysis as an outlier, one litter in the 1,125 ppm group had no live fetuses, and one litter in the 1,125 ppm group had no live male fetuses.

^dStatistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

^eNo females were confirmed pregnant for the 3,750 ppm group.

^fStatistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^gBecause a positive trend in fetal weight with litter size was seen in all exposure groups, only unadjusted fetal weights are presented here.

^hBody weight adjusted for gravid uterus weight.

Fetal Findings

Placental Morphology

There was no effect of BPAF exposure on the incidence of gross placental abnormalities (Appendix E). Retained placentae was noted for a single litter of an F₀ female in the 3,750 ppm group.

External

There was no effect of BPAF exposure on the incidence of fetal external abnormalities (Appendix E), which were limited to a single fetus in the 338 ppm group that displayed a clubbed hind limb.

Visceral

There was no effect of BPAF exposure on the incidence of fetal visceral abnormalities. Distended ureter (a variation found in 7%, 11%, and 12% of fetuses and 44%, 45%, and 43% of litters for the control, 338, and 1,125 ppm groups, respectively) and hydroureter (a malformation found in 0.4%, 1%, and 2% of fetuses and 6%, 10%, and 7% of litters for the control, 338, and 1,125 ppm groups, respectively) were noted in several animals. There is a relatively high background incidence of abnormalities associated with the kidney and ureter in this strain of rat, however, and these values were not outside of NTP historical control data (distended ureter—4.83% to 15.36% for fetuses and 43.75% to 68.18% for litters; hydroureter—0.17% to 2.83% for fetuses and 2.27% to 21.05% for litters) (Appendix E).

Other visceral findings (i.e., dilated renal pelvis, agenesis of the innominate artery, and hydronephrosis) were limited to one or two occurrences or were found in the control group and, therefore, were not considered exposure related.

Head

Fetal head abnormalities noted in the 1,125 ppm group were attributed to BPAF exposure. Four pups from four litters had dilated lateral ventricles (variation), and one pup also presented with a misshapen lateral ventricle (variation) in the brain (Table 27). NTP has not recorded either finding in its previous studies and, therefore, these abnormalities are outside of NTP's historical control range. No other findings were noted.

Table 27. Summary of Head Findings in Fetuses Exposed to Bisphenol AF in Feed

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. Litters Examined	16	19	13	— ^a
No. Fetuses Examined	117	127	48	—
Head ^{b,c}				
Dilated lateral ventricle, bilateral – [V] ^d				
Fetuses	0 (0.00)	0 (0.00)	4 (8.33)	—
Litters	0 (0.00)	0 (0.00)	4 (30.77)	—
Misshapen lateral ventricle, left – [V] ^d				
Fetuses	0 (0.00)	0 (0.00)	1 (2.08)	—
Litters	0 (0.00)	0 (0.00)	1 (7.69)	—

[V] = variation.

^aNo females were confirmed pregnant for the 3,750 ppm group.

^bUpper row denotes number of affected fetuses (%) and lower row the number of affected litters (%).

^cStatistical analysis for fetal data including litter effects was performed using a Rao-Scott modification to the Cochran-Armitage test where the litter was the random effect for both trend and pairwise analyses.

^dHistorical control incidence for all routes: fetuses – 0/691 (0.00%); litters – 0/97 (0.00%).

Skeletal

There was a slight increase in the incidence of rudimentary lumbar I ribs in fetuses in the 338 and 1,125 ppm groups and in the incidence of full lumbar I ribs in the 338 ppm fetuses compared to the control group (Table 28). Skeletal abnormalities in exposed groups were limited to the lumbar rib (rudimentary and full) findings, incomplete ossification of the sternbrae, and bipartite and dumbbell ossification of the thoracic centrum. With the exception of the lumbar rib observations, findings were observed only in a single fetus. Rudimentary ribs (variation) were defined as ribs that were shorter than half the length of the 13th rib. Ribs that were longer than half the length of the 13th rib were considered full (malformation). The incidences of rudimentary lumbar ribs and full lumbar ribs (Table 28) were slightly outside of NTP historical control data (Table 28). While these findings might have been related to BPAF exposure, the lack of an exposure-related response for rudimentary ribs in litters and the absence of full lumbar ribs in the 1,125 ppm group impede the evaluation. These issues could be due to the low number of fetuses in the 1,125 ppm group (102 fetuses compared to 234 and 265 fetuses in the control group and 338 ppm group, respectively).

Table 28. Summary of Select Skeletal Findings in Fetuses Exposed to Bisphenol AF in Feed

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. Litters Examined	16	20	14	— ^a
No. Fetuses Examined	234	265	102	—
Ribs ^{b,c}				
Lumbar I, rudimentary, total – [V] ^d				
Fetuses	11 (4.70)	19 (7.17)	14 (13.73)	—
Litters	6 (37.50)	10 (50.00)	4 (28.57)	—
Lumbar I, full, total – [M] ^e				
Fetuses	0 (0.00)	4 (1.51)	0 (0.00)	—
Litters	0 (0.00)	3 (15.00)	0 (0.00)	—

[V] = variation; [M] = malformation.

^aNo females were confirmed pregnant for the 3,750 ppm group.

^bUpper row denotes number of affected fetuses (%) and lower row the number of affected litters (%).

^cStatistical analysis for fetal data including litter effects was performed using a Rao-Scott modification to the Cochran-Armitage test where the litter was the random effect for both trend and pairwise analyses.

^dHistorical control incidence: fetuses – 114/1,385 (8.23%), range 3.35%–13.69%; litters – 53/97 (54.64%), range 26.32%–65.91%.

^eHistorical control incidence: fetuses – 4/1,385 (0.29%), range 0.00%–0.67%; litters – 4/97 (4.12%), range 0.00%–9.09%.

Reproductive Performance Cohort Findings

F₁ and F₂ rats from the reproductive performance cohort were evaluated for maternal reproductive performance and offspring effects, respectively, as shown in Figure 28. Littering, mean body weights, and feed consumption results from the F₁ rats as well as viability, clinical observations, mean body weights, and gross pathology results from the F₂ rats are presented below.

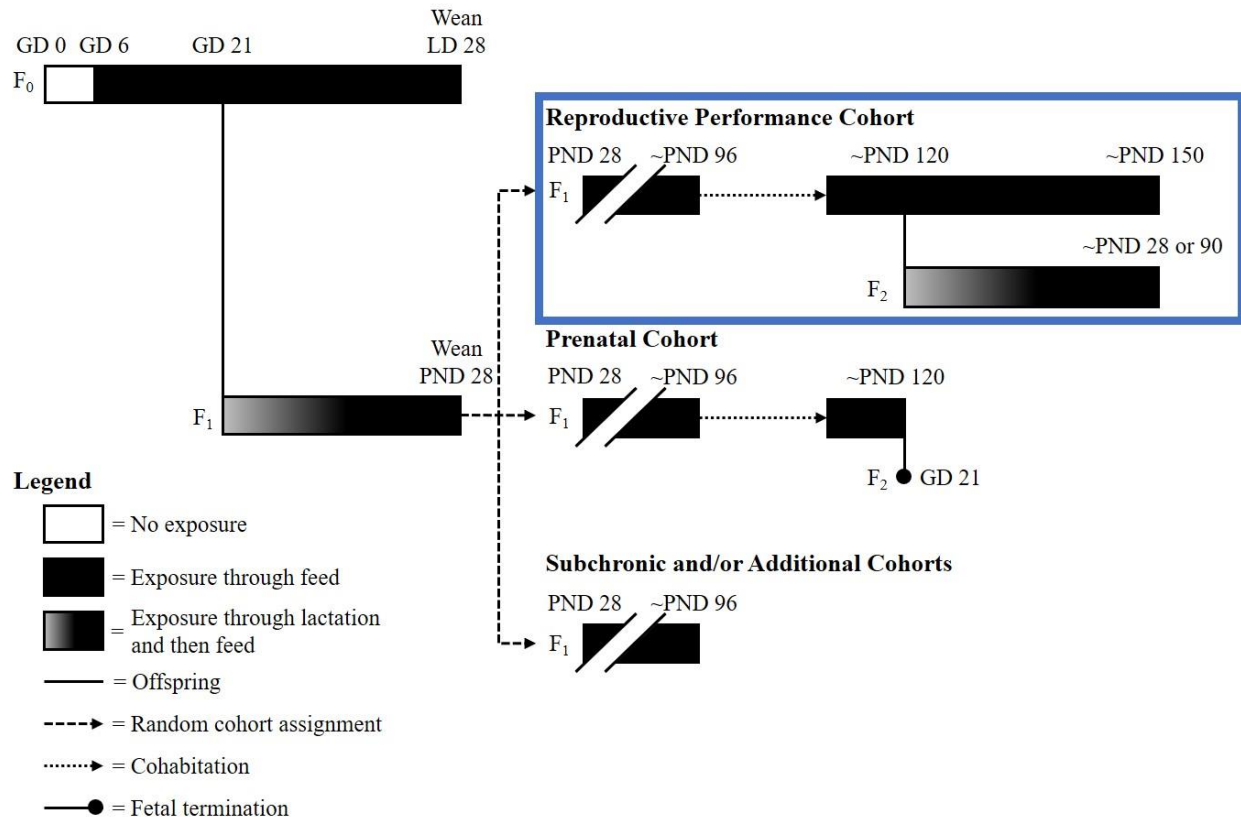


Figure 28. Design of the Modified One-Generation Study – Reproductive Performance Cohort

GD = gestation day; LD = lactation day; PND = postnatal day.

Reproductive Performance and Littering

Reproductive performance and littering parameters for the reproductive performance cohort are presented in Table 29. Gestation length was similar for dams in the 338 and 1,125 ppm groups and the control group (no 3,750 ppm F₂ generation was produced for either the prenatal or reproductive performance cohorts). Significant exposure-related decreases in mean live litter size on LD 0 (by approximately five pups) were observed in the 1,125 ppm group (Appendix E). This decrease continued after litter standardization on LD 4 (with a difference of approximately two pups) through LD 28 (Appendix E). These findings were consistent with the significant decreases in the mean number of live fetuses per litter (decrease of approximately seven pups) that were observed in the prenatal cohort (Table 26).

Table 29. Summary of Reproductive Parameters of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. Females Paired	22	23	21	19
No. Females Mated	22	23	16	1
No. Pregnant Females	18	22	12	0
No. Females Littering	18	20	9	0
Percent of Mated Females/Paired ^{a,b}	100.0**	100.0	76.2*	5.3**
Percent of Littered Females/Paired ^{a,b}	81.8**	87.0	42.9*	0.0**
Percent of Pregnant Females/Mated ^{a,b}	81.8*	95.7	75.0	0.0
Percent of Littered Females/Mated ^{a,b}	81.8**	87.0	56.3	0.0
Precoital Interval (days) ^{c,d,e}	6.4 ± 0.7* (20)	5.3 ± 0.9 (20)	4.1 ± 1.2 (13)	1.0 ± 0.0 (1)
Gestation Length (days) ^{c,d,f}	22.6 ± 0.1 (16)	22.7 ± 0.1 (17)	23.3 ± 0.6 (7)	— ^g

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aStatistical analysis performed using the Cochran-Armitage (trend) and Fisher's exact (pairwise) test comparisons.

^bAnimals removed from the study between mating and littering were excluded from calculations of % littered females.

^cStatistical analysis performed using the Jonckheere (trend) and the Shirley or Dunn (pairwise) tests.

^dData are displayed as mean ± standard error (n).

^ePrecoital interval calculated for sperm-positive females.

^fGestation length calculated for sperm-positive females that delivered a litter.

^gNo females were confirmed pregnant for the 3,750 ppm group.

Lactation Body Weights and Feed Consumption

Consistent with their pre-mating and gestation weights, F₁ female mean body weights during lactation were significantly decreased in both the 338 and 1,125 ppm groups relative to the control group (Table 30; Figure 29). For the 338 ppm group, on LDs 1 and 28, female mean body weights were significantly decreased by 10% and 8%, respectively, compared to the control group; for the 1,125 ppm group, female mean body weights were significantly decreased by 21% and 13% on LDs 1 and 28, respectively. Mean body weight gain over the LD 4–28 interval in the 1,125 ppm group was significantly increased relative to the control group. In general, relative feed consumption values (g/kg/day) during lactation in the groups exposed to BPAF were similar to the control group (Table 30). BPAF intakes during lactation in the 338 and 1,125 ppm groups, based on feed consumption and dietary concentrations for LD 1–13, were approximately 53 and 162 mg/kg/day, respectively (Table 30).

Table 30. Summary of Mean Body Weights, Body Weight Gains, and Feed and Test Article Consumption of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed during Lactation

Lactation Day ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Body Weight (g)^b				
1	307.6 ± 5.0** (18)	276.6 ± 5.6** (19)	243.0 ± 7.7** (9)	— ^c
13	326.7 ± 4.6** (18)	299.6 ± 4.8** (19)	269.8 ± 5.6** (9)	—
28	305.4 ± 3.7** (18)	281.1 ± 3.8** (19)	264.6 ± 6.9** (9)	—

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Lactation Day ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Body Weight Gain (g)^b				
4–28	-8.5 ± 2.8** (18)	-7.7 ± 2.3 (19)	16.1 ± 4.8** (9)	–
Feed Consumption^b				
1–13 (g/animal/day)	44.9 ± 1.6 (18)	45.8 ± 0.9 (19)	37.0 ± 4.0 (9)	–
1–13 (g/kg/day)	142.2 ± 5.4 (18)	158.1 ± 3.4 (19)	144.3 ± 16.0 (9)	–
Chemical Intake (mg/kg/day)^{d,e}				
1–13	0.0 ± 0.0 (18)	53.4 ± 1.2 (19)	162.4 ± 18.0 (9)	–

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

^aData are displayed as mean ± standard error (n), where n = number of animals. Feed consumption values were excluded when excessive spillage was recorded. Changes in n are the result of removing litters with no surviving pups by lactation day 26 (one dam in the 338 ppm group).

^bStatistical analysis performed using the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^cNo females were confirmed pregnant for the 3,750 ppm group.

^dChemical intake calculated as: $([\text{exposure concentration} \times \text{feed consumption}]/[\text{average body weight of day range}])$.

^eNo statistical analysis performed on the chemical intake data.

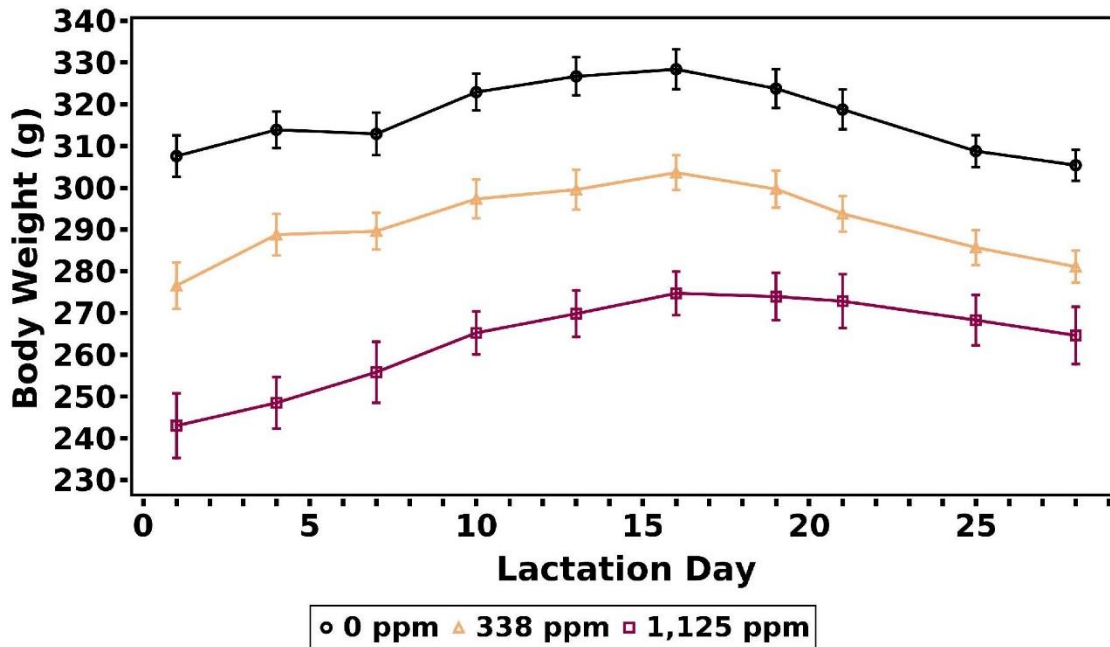


Figure 29. Lactation Growth Curves for F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed

Information for statistical significance in maternal weights is provided in Table 30.

F₂ Viability and Clinical Observations

There was no effect of BPAF exposure on pup survival (Table 31). There were no clinical observations in the F₂ pups attributed to BPAF exposure. Clinical observations noted in individual pups in all exposure groups, including the control group, were typically indicative of an individual pup not thriving (e.g., cold to touch, no milk in stomach) (Appendix E).

Table 31. Summary of F₂ Litter Size and Pup Survival Following Perinatal Exposure to Bisphenol AF

Postnatal Day	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. of Live Pups (Litters)^a				
0	201 (18)	211 (20)	58 (9)	— ^b
Total Litter Size^{c,d}				
0	11.7 ± 1.1 (18)	12.0 ± 0.7 (20)	7.4 ± 1.6 (9)	—
Live Litter Size^{c,d}				
0	11.2 ± 1.0* (18)	10.6 ± 0.8 (20)	6.4 ± 1.4* (9)	—
1	10.9 ± 1.0* (18)	11.1 ± 0.6 (19) ^e	6.4 ± 1.4* (9)	—
4 (prestandardization)	10.9 ± 1.0* (18)	11.1 ± 0.6 (19)	6.4 ± 1.4* (9)	—
4 (poststandardization)	7.3 ± 0.4 (18)	7.9 ± 0.1 (19)	5.7 ± 1.1 (9)	—
7	7.3 ± 0.4 (18)	7.8 ± 0.1 (19)	5.7 ± 1.1 (9)	—
13	7.2 ± 0.4 (18)	7.7 ± 0.1 (19)	5.2 ± 1.1 (9)	—
21	7.2 ± 0.4 (18)	7.7 ± 0.1 (19)	5.2 ± 1.1 (9)	—
28	7.2 ± 0.4 (18)	7.7 ± 0.1 (19)	5.2 ± 1.1 (9)	—
No. of Dead Pups (Litters)^a				
0	10 (7)	28 (15)	9 (4)	—
1–4	4 (3)	1 (1)	0 (0)	—
5–28	1 (1)	3 (2)	4 (1)	—
Dead/Litter^{c,d}				
0	0.56 ± 0.20 (18)	1.40 ± 0.29* (20)	1.00 ± 0.47 (9)	—
1–4	0.22 ± 0.13 (18)	0.05 ± 0.05 (20)	0.00 ± 0.00 (9)	—
5–28	0.06 ± 0.06 (18)	0.16 ± 0.12 (19) ^e	0.44 ± 0.44 (9)	—
Survival Ratio^{c,d}				
0	0.96 ± 0.02 (18)	0.87 ± 0.04* (20)	0.87 ± 0.06 (9)	—
1–4	0.97 ± 0.02 (18)	0.95 ± 0.05 (20)	1.00 ± 0.00 (9)	—
5–28	0.99 ± 0.01 (18)	0.98 ± 0.01 (19) ^e	0.94 ± 0.06 (9)	—

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$.

^an = the number of pups examined (number of F₁ litters). For no. of dead pups, n is the number of litters contributing dead pups.

^bNo females were confirmed pregnant for the 3,750 ppm group.

^cData are displayed as the mean of litter values ± standard error of litter values (n = number of litters contributing).

^dStatistical analysis performed using the Jonckheere (trend) and Shirley or Dunn (pairwise) tests. All calculations were based on the last litter observation of the day.

^eChanges in n are the result of removing litters with no surviving pups by postnatal day 1 (one F₁ litter in the 338 ppm group).

F₂ Body Weights and Feed Consumption

Male Pups

Male pups exposed to 1,125 ppm BPAF had significantly decreased preweaning mean body weights (litter means) over time compared to the control group (Table 32; Figure 30; Appendix E). On PND 28, male pup mean body weights per litter in the 1,125 ppm group were significantly decreased by approximately 12% relative to the control group. Significant decreases in pup mean body weights occurred at select time points throughout the postnatal period (PNDs 4, 16, 21, 25, and 28) with most occurring toward the end of the weaning period (Appendix E). The magnitude of effect is consistent with what was observed in the F₁ generation (12% decrease in preweaning mean body weight on PND 28). Pup mean body weights of the 338 ppm group were within 5%–6% below the control values at all time points between PND 1 and PND 28 (Appendix E).

Postweaning F₂ male mean body weights were lower compared to the control group from PND 28 through PND 91 in the 1,125 ppm group (Table 33; Figure 31). The lower body weights were associated with a significant decrease in absolute, but not relative, feed consumption, suggesting that changes in absolute feed consumption may be related to the size of the animals. BPAF intakes by F₂ males, based on feed consumption and dietary concentrations for PND 28–91, were 28 and 94 mg/kg/day at 338 and 1,125 ppm, respectively.

Female Pups

Female pups exposed to 1,125 ppm BPAF also displayed significantly decreased preweaning mean body weights (litter means) relative to the control group (Table 32; Figure 32; Appendix E). On PND 28, female pup mean body weights per litter in the 1,125 ppm group were significantly decreased by approximately 12%. This effect is consistent with what was observed in the F₁ generation, although the difference from the control group was greater for the F₂ generation early in the postnatal period. Pup mean body weights of the 338 ppm group were no more than 7% below the control values for all time points between PND 1 and PND 28 (Appendix E).

Significant decreases in postweaning F₂ female mean body weights continued through PND 91 in the 1,125 ppm group (Table 33; Figure 33). The decreased body weights were associated with lower absolute, but significantly increased relative, feed consumption, suggesting that changes in absolute feed consumption may be related to the size of the animals. BPAF intakes by F₂ females, based on feed consumption and dietary concentrations for PND 28–91, were 32 and 108 mg/kg/day at 338 and 1,125 ppm, respectively.

Table 32. Summary of F₂ Male and Female Pup Mean Body Weights and Body Weight Gains Following Perinatal Exposure to Bisphenol AF^{a,b}

Postnatal Day	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Male				
Body Weight				
1	7.32 ± 0.15 79 (17) ^c	7.00 ± 0.16 108 (19)	6.80 ± 0.25 32 (7)	— ^d
4	11.13 ± 0.24** 79 (17)	10.47 ± 0.29 108 (19)	9.41 ± 0.67* 32 (7)	—
21	53.41 ± 1.42* 52 (17)	52.93 ± 0.98 70 (19)	47.03 ± 2.54* 27 (7)	—
28	88.86 ± 2.01** 52 (17)	86.96 ± 1.49 70 (19)	77.82 ± 4.07* 27 (7)	—
Body Weight Gain ^e				
4–28	77.63 ± 1.64** 52 (17)	76.12 ± 1.20 70 (19)	67.07 ± 3.63** 27 (7)	—
Female				
Body Weight				
1	7.15 ± 0.15** 118 (18)	6.79 ± 0.14 102 (19)	6.28 ± 0.32* 26 (7)	—
4	10.77 ± 0.24** 118 (18)	10.06 ± 0.24 102 (19)	8.54 ± 0.67** 26 (7)	—
21	51.36 ± 1.11** 78 (18)	50.35 ± 0.79 77 (19)	44.84 ± 2.28** 20 (7)	—
28	81.62 ± 1.31** 78 (18)	78.23 ± 1.15 77 (19)	71.69 ± 2.52** 20 (7)	—
Body Weight Gain ^e				
4–28	70.82 ± 1.09** 78 (18)	67.83 ± 0.94 77 (19)	62.17 ± 1.98** 20 (7)	—

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData are displayed as mean ± standard error of the litter means. Body weights are presented in grams.

^bStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons. Pup weights were adjusted for covariate litter size: total live on postnatal day 1 for day 1 to the day of standardization and number of live pups poststandardization for later days.

^cn = number of pups examined (number of F₁ litters). One litter in the vehicle control group had no male pups.

^dNo females were confirmed pregnant for the 3,750 ppm group.

^eBody weight gain (data are presented in grams).

Table 33. Summary of Postweaning Mean Body Weights, Body Weight Gains, and Feed and Test Article Consumption of All F₂ Male and Female Rats Exposed to Bisphenol AF in Feed

Postnatal Day ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Male				
Body Weight (g) ^{b,c}				
28	87.5 ± 2.5* 52 (17)	85.8 ± 1.6 70 (19)	78.6 ± 4.1 27 (7)	— ^d
91	387.9 ± 6.7* 52 (17)	372.4 ± 5.3 70 (19)	360.2 ± 9.8 27 (7)	—
Body Weight Gain (g) ^{b,c}				
28–91	300.4 ± 5.8 52 (17)	286.6 ± 4.5 70 (19)	281.6 ± 7.3 27 (7)	—
Postweaning Feed Consumption ^{e,f}				
28–91 (g/animal/day)	22.0 ± 0.2** (24)	21.5 ± 0.2 (34)	20.7 ± 0.4** (13)	—
28–91 (g/kg/day)	83.9 ± 1.2 (24)	83.1 ± 0.7 (34)	83.8 ± 1.3 (13)	—
Chemical Intake (mg/kg/day) ^{f,g,h}				
28–91	0.0 ± 0.0 (24)	28.1 ± 0.2 (34)	94.2 ± 1.4 (13)	—
Female				
Body Weight (g)				
28	81.1 ± 1.7** 78 (18)	76.8 ± 1.2 77 (19)	73.9 ± 1.9* 20 (7)	—
91	240.3 ± 4.2** 78 (18)	217.6 ± 4.0** 77 (19)	203.9 ± 5.9** 20 (7)	—
Body Weight Gain (g)				
28–91	159.2 ± 3.6** 78 (18)	140.8 ± 3.9** 77 (19)	130.0 ± 5.9** 20 (7)	—
Postweaning Feed Consumption				
28–91 (g/animal/day)	16.0 ± 0.2* (37)	15.4 ± 0.3 (37)	14.8 ± 0.6 (10)	—
28–91 (g/kg/day)	88.7 ± 0.7** (37)	94.1 ± 1.4** (37)	96.4 ± 2.9* (10)	—
Chemical Intake (mg/kg/day)				
28–91	0.0 ± 0.0 (37)	31.8 ± 0.5 (37)	108.4 ± 3.2 (10)	—

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData are displayed as mean ± standard error (n). Feed consumption values were excluded when excessive spillage was recorded.

^bStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

^cn = number of pups examined (number of F₁ litters). One litter in the vehicle control group had no male pups.

^dNo females were confirmed pregnant for the 3,750 ppm group.

^eStatistical analysis performed using the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^fn = number of cages.

^gChemical intake calculated as: $([\text{exposure concentration} \times \text{feed consumption}]/[\text{average body weight of day range}])$.

^hNo statistical analysis performed on the chemical intake data.

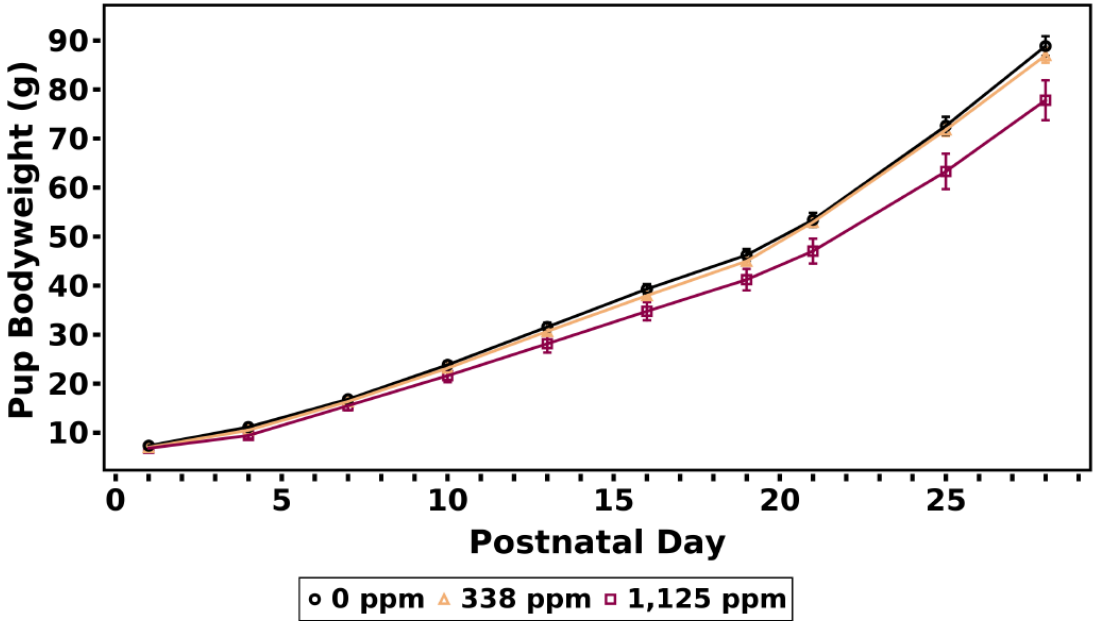


Figure 30. Lactation Growth Curves for F₂ Male Pups Following Perinatal Exposure to Bisphenol AF

Information for statistical significance in F₂ male rat weights is provided in Table 32.

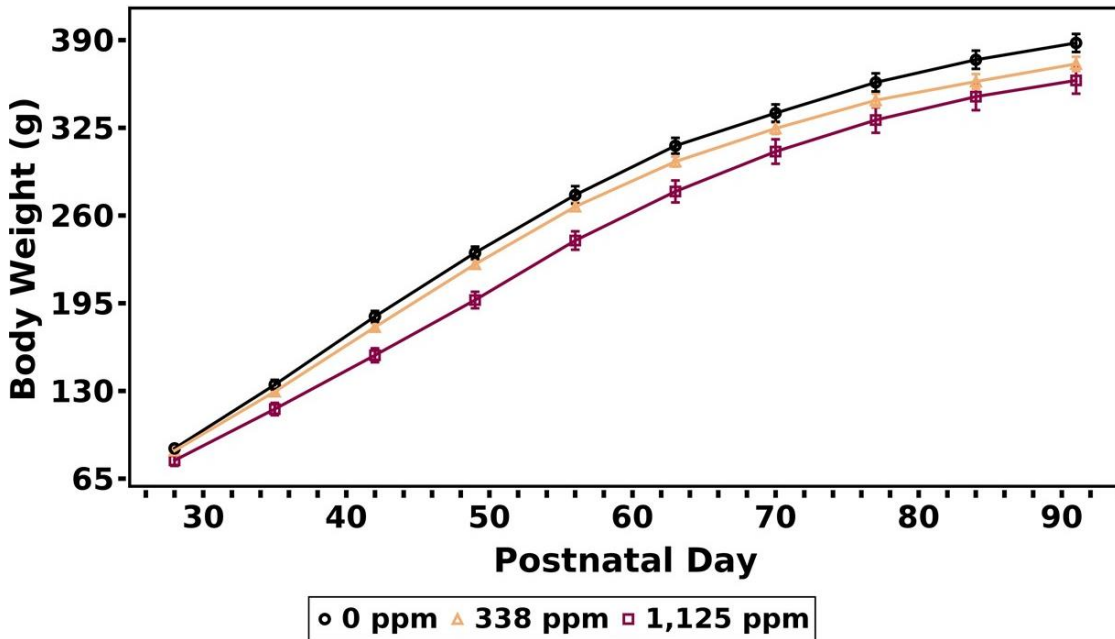


Figure 31. Postweaning Growth Curves for All F₂ Male Rats Exposed to Bisphenol AF in Feed

Information for statistical significance in F₂ male rat weights is provided in Table 33.

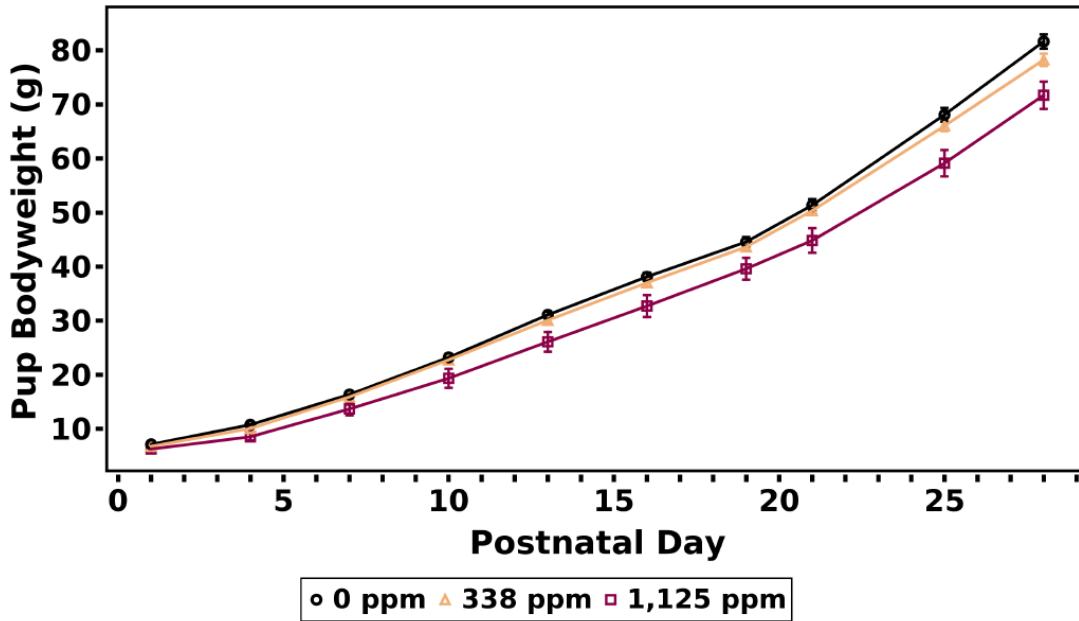


Figure 32. Lactation Growth Curves for F₂ Female Pups Following Perinatal Exposure to Bisphenol AF

Information for statistical significance in F₂ female rat weights is provided in Table 32.

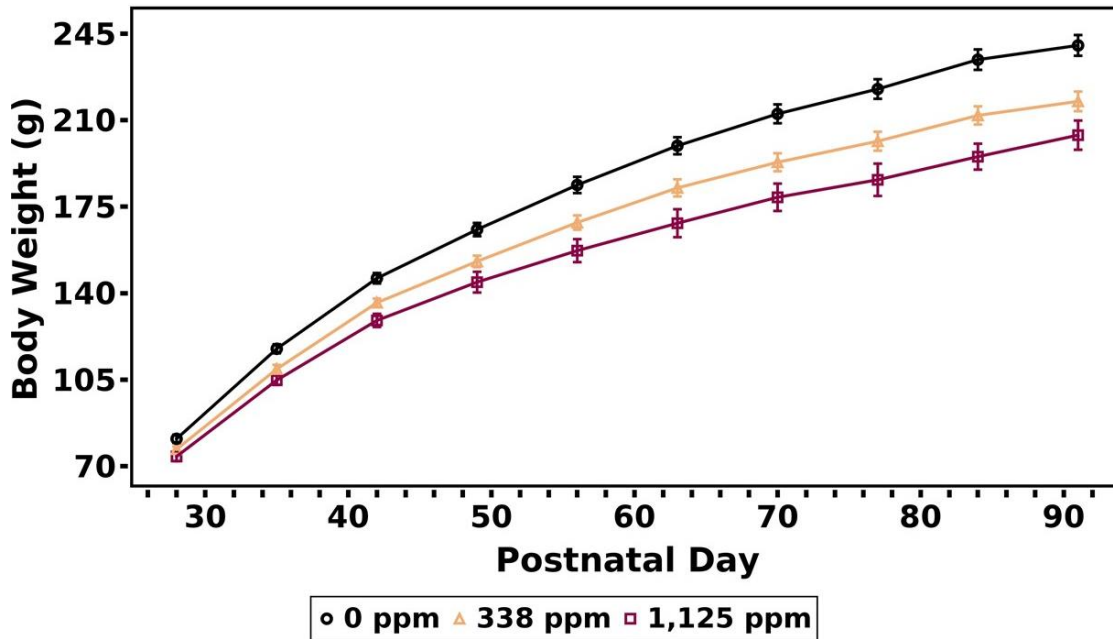


Figure 33. Postweaning Growth Curves for All F₂ Female Rats Exposed to Bisphenol AF in Feed

Information for statistical significance in F₂ female rat weights is provided in Table 33.

F₁ Necropsies: Prenatal, Reproductive Performance, and Subchronic Cohorts

Male Necropsies

The F₁ males in the reproductive performance cohort were euthanized at 152–154 days of age, following completion of littering of the F₂ generation. F₁ males in the prenatal and subchronic cohorts were euthanized following completion of pairing at 119–121 and 115–119 days of age, respectively. There were BPAF-related gross findings, including two F₁ males with malformations of the penis in the 3,750 ppm group: one male with the os penis visible at the glans and a second male with incomplete BPS (Table 34, Table 35). Both males also had a reduced size of the dorsolateral prostate, ventral prostate, seminal vesicles, testes, and epididymides. Terminal body (necropsy) weights of male rats exposed to 1,125 and 3,750 ppm BPAF for the three cohorts were significantly decreased by 13%–14% and 34%–40%, respectively, relative to the control males (Table 36, Table 37).

There was a BPAF-related significant increase in the relative weights of the adrenal glands and thyroid in the F₁ males in the subchronic cohort (Table 36). Although absolute adrenal and thyroid gland weights were similar to the control group for the 3,750 ppm group, relative weights were significantly increased, indicating that the adrenals and thyroid were large relative to the size of the animals. There was no effect of BPAF exposure on weights of the adrenal and thyroid glands for the 338 and 1,125 ppm groups.

Absolute weight of the lungs in the 3,750 ppm group of the subchronic cohort was significantly decreased to 21% below the control group, whereas relative weight of the lungs for the 3,750 ppm group was significantly increased (Table 36). There was no effect of BPAF exposure on weight of the lungs for the 338 and 1,125 ppm groups.

For F₁ males in the subchronic cohort, the absolute liver, kidney (left and right), heart, and thymus weights were significantly decreased to 12%–24% and 29%–38% less than the control group for the 1,125 and 3,750 ppm groups, respectively (Table 36). Relative organ weights were not significantly different from the control group for the right kidney and thymus, suggesting that the lower absolute weights for these tissues might have been secondary to the effect of BPAF on body weight. There were significant decreases in relative liver and left kidney weights and a positive trend for relative heart weight for the 3,750 ppm group. There was no effect of BPAF exposure on liver, kidney (left and right), heart, and thymus weights for the 338 ppm group.

Absolute weights of the dorsolateral prostate for F₁ males from the 3,750 ppm groups across the three cohorts were significantly decreased by 56%–68% below the respective control groups (Table 36, Table 37). Absolute weights of the ventral prostate across the three cohorts were significantly decreased by 14%–26% and 69%–76% below that of the control group for the 1,125 and 3,750 ppm groups, respectively. Relative weights of the dorsolateral and ventral prostate were significantly decreased for the 3,750 ppm groups across the three cohorts; relative weights for the ventral prostate were also significantly decreased for the 1,125 ppm groups in the subchronic and prenatal cohorts. The magnitudes of the reduction in weights of the dorsolateral prostate in the 3,750 ppm groups and the ventral prostate in the 1,125 and 3,750 ppm groups were more than the magnitudes of the reduction in body weights, suggesting a direct BPAF-mediated suppression of maturation of these tissues. The decrease in absolute weight of the dorsolateral prostate for the 1,125 ppm group was only significant (16%) for the prenatal cohort,

although there was also a similar degree of reduction in weight of the dorsolateral prostate (13.6%) in the subchronic cohort, and might have been secondary to the effect of BPAF on body weight. There was no effect of BPAF exposure on prostate weight for the 338 ppm group.

Absolute weights of the seminal vesicles with coagulating glands for F₁ males across the three cohorts were lower by 10%–13% compared to the control group (significant in the prenatal and reproductive performance cohorts) in the 1,125 ppm groups and significantly decreased by 70%–80% compared to the control group for the 3,750 ppm groups (Table 36, Table 37). Relative weight of the seminal vesicles with coagulating glands for males in the 3,750 ppm group was significantly decreased. The magnitude of the reduction in weight of the seminal vesicles for the 3,750 ppm group was more than the magnitude of the reduction in body weight, suggesting a direct BPAF-mediated suppression of maturation of this tissue. The changes in absolute weight of the seminal vesicles for the 1,125 ppm group might have been secondary to the effect of BPAF on body weight given that the corresponding relative weight was similar to that of the control group. There was no effect of BPAF exposure on seminal vesicle weight for the 338 ppm group.

The levator ani/bulbocavernosus muscle (LABC) and Cowper's glands were weighed in both the prenatal and reproductive performance cohorts (Table 37). Absolute weights of the LABC were significantly decreased by 7%–12% and 60%–62% compared to the control group for the 1,125 and 3,750 ppm groups, respectively. Relative weights were significantly decreased for the 3,750 ppm group. Absolute weights of the Cowper's glands were significantly decreased by 17% (reproductive performance cohort only), 16%–17%, and 66%–67% compared to the control group for the 338, 1,125, and 3,750 ppm groups, respectively. Relative weights were significantly decreased for the 3,750 ppm group. The magnitude of the reductions in weights of the LABC and the Cowper's glands for the 3,750 ppm group were more than the magnitude of the reduction in body weight, suggesting a direct BPAF-mediated suppression of maturation of these tissues. The reductions in absolute weights in the 338 (Cowper's glands only) and 1,125 ppm groups (LABC and Cowper's glands) might have been secondary to the effects of BPAF on body weight given that the corresponding relative weights were not significantly different from the control group. There was no effect of BPAF exposure on weights of the LABC for the 338 ppm group.

For F₁ males across the three cohorts, absolute testis weights (right and left) were lower by 5%–12% and significantly decreased by 25%–33% below the control group for the 1,125 and 3,750 ppm groups, respectively (Table 36, Table 37). Absolute weights of the epididymides were lower by 7%–8% in the subchronic cohort and significantly decreased by 8%–12% in the prenatal and reproductive performance cohorts in the 1,125 ppm group. Absolute weights of the epididymides were significantly decreased by 33%–42% compared to the control group in the 3,750 ppm group. Although relative testis weights were higher for the 1,125 and 3,750 ppm groups and suggest that the lower absolute weights might have been secondary to the effect of BPAF on body weight, the histopathological findings in these tissues indicate a potential direct impact of exposure to BPAF (Appendix E). Relative epididymis weights were similar to the control group. The testicular weight changes in the 3,750 ppm group correlated with a significant increase of testis spermatid head concentration (24% above the control group). The significant decrease in absolute epididymal weight for the 3,750 ppm group compared to the control group correlated with a reduction in cauda epididymal sperm concentration (17% below the control

group). There was a significant decrease of absolute testis (left) and epididymis (left) weights for the animals in the 338 ppm prenatal cohort.

The preputial glands were weighed in both the prenatal and reproductive performance cohorts (Table 37). Absolute weight of the preputial glands in the 3,750 ppm group was significantly decreased by 32%–35% below the control group. Relative weight of the preputial glands was not significantly different from the control group, suggesting that the lower absolute weight in the 3,750 ppm group might have been secondary to the effect of BPAF on body weight. There was no effect of BPAF exposure on the weight of the preputial glands for the 338 and 1,125 ppm groups.

Table 34. Summary of Gross Necropsy Findings in Adult F₁ Male Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed^a

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. of Animals Examined ^b	10 (10)	10 (10)	10 (10)	10 (10)
Prostate Gland				
Reduced ^c	0**	0	0	10 (10)**
Seminal Vesicles				
Reduced				
Bilateral	0**	0	0	10 (10)**
Phallus				
Deformity	0	0	0	1 (1)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

^aStatistical analysis performed using the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.

^bNumber of animals (number of litters) examined for gross lesions.

^cNumber of animals (number of litters) with lesion.

Table 35. Summary of Gross Necropsy Findings in Adult F₁ Male Rats in the Prenatal and Reproductive Performance Cohorts Exposed to Bisphenol AF in Feed^{a,b}

	0 ppm		338 ppm		1,125 ppm		3,750 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC
No. of Animals Examined ^c	22 (22)	22 (22)	24 (24)	21 (21)	21 (21)	22 (22)	20 (20)	20 (20)
Cowper's Gland								
Missing								
Left ^d	0	0	0	0	0	1 (1)	0	0
Bilateral	0*	0*	0	0	0	0	2 (2)	2 (2)
Total	0*	0	0	0	0	1 (1)	2 (2)	2 (2)
Reduced								
Left	0	0	1 (1)	0	0	0	0	0
Bilateral	0**	0**	1 (1)	0	0	0	14 (14)**	14 (14)**
Total	0**	0**	2 (2)	0	0	0	14 (14)**	14 (14)**

Bisphenol AF, NTP DART 08

	0 ppm		338 ppm		1,125 ppm		3,750 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC
Levator Ani/bulbocavernosus Muscle Complex								
Reduced	0**	0**	0	0	0	0	16 (16)**	18 (18)**
Dorsolateral Prostate Gland ^e								
Reduced	0**	–	0	–	0	–	18 (18)**	–
Ventral Prostate Gland								
Reduced	0**	–	0	–	0	–	18 (18)**	–
Prostate Gland								
Reduced	–	0**	–	0	–	0	–	20 (20)**
Seminal Vesicles								
Reduced								
Left	0	0	0	0	0	1 (1)	0	0
Right	0	0	0	1 (1)	0	0	0	0
Bilateral	0**	0**	0	0	0	0	18 (18)**	20 (20)**
Total	0**	0**	0	1 (1)	0	1 (1)	18 (18)**	20 (20)**
Phallus								
Misshapen	0	0	0	0	0	0	1 (1)	0

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

RPC = reproductive performance cohort; PC = prenatal cohort.

^aData for the RPC and PC are also presented separately by cohort in Appendix E.

^bStatistical analysis performed using the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.

^cNumber of animals (number of litters) examined for gross lesions.

^dNumber of animals (number of litters) with lesion.

^eRPC necropsy findings for the prostate gland are distinguished between dorsalateral and ventral prostate gland. PC necropsy findings are presented for the prostate gland overall, without distinction.

Table 36. Summary of Organ Weights of Adult F₁ Male Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed^{a,b}

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. of Litters Examined	10	10	10	10
Necropsy Body Wt. (g)	399.6 ± 7.1**	409.6 ± 12.6	347.7 ± 8.6**	263.6 ± 6.8**
Adrenal Glands				
Absolute (g)	0.0633 ± 0.0044	0.0537 ± 0.0050	0.0531 ± 0.0041	0.0605 ± 0.0033
Relative (mg/g) ^c	0.16 ± 0.01**	0.13 ± 0.01	0.15 ± 0.01	0.23 ± 0.01**
Thyroid Gland				
Absolute (g)	0.0171 ± 0.0008	0.0199 ± 0.0014	0.0175 ± 0.0015	0.0162 ± 0.0009
Relative (mg/g)	0.04 ± 0.00**	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00**
Lung				
Absolute (g)	2.13 ± 0.06**	2.25 ± 0.10	1.89 ± 0.09	1.69 ± 0.08**
Relative (mg/g)	5.33 ± 0.15*	5.50 ± 0.20	5.44 ± 0.18	6.44 ± 0.30**

Bisphenol AF, NTP DART 08

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Liver				
Absolute (g)	16.03 ± 0.26**	16.42 ± 0.87	13.59 ± 0.49**	9.90 ± 0.33**
Relative (mg/g)	40.15 ± 0.41**	39.90 ± 1.18	39.01 ± 0.55	37.53 ± 0.71*
Right Kidney				
Absolute (g)	1.43 ± 0.02**	1.47 ± 0.05	1.22 ± 0.04**	0.91 ± 0.03**
Relative (mg/g)	3.60 ± 0.04	3.58 ± 0.07	3.52 ± 0.06	3.45 ± 0.07
Left Kidney				
Absolute (g)	1.43 ± 0.02**	1.46 ± 0.04	1.19 ± 0.04**	0.88 ± 0.03**
Relative (mg/g)	3.59 ± 0.05**	3.56 ± 0.05	3.42 ± 0.07	3.33 ± 0.06**
Heart				
Absolute (g)	1.47 ± 0.03**	1.47 ± 0.05	1.29 ± 0.04**	1.04 ± 0.03**
Relative (mg/g)	3.68 ± 0.11*	3.59 ± 0.08	3.70 ± 0.05	3.93 ± 0.06
Thymus				
Absolute (g)	0.400 ± 0.032** ^d	0.400 ± 0.021	0.304 ± 0.027*	0.262 ± 0.025**
Relative (mg/g)	0.99 ± 0.07 ^d	0.97 ± 0.04	0.87 ± 0.07	1.00 ± 0.10
Dorsolateral Prostate Gland				
Absolute (g)	0.450 ± 0.021**	0.510 ± 0.054 ^d	0.389 ± 0.017	0.200 ± 0.019** ^d
Relative (mg/g)	1.13 ± 0.06**	1.23 ± 0.12 ^d	1.13 ± 0.07	0.75 ± 0.07** ^d
Ventral Prostate Gland				
Absolute (g)	0.610 ± 0.032**	0.578 ± 0.036	0.451 ± 0.021**	0.188 ± 0.023** ^d
Relative (mg/g)	1.53 ± 0.08**	1.41 ± 0.07	1.30 ± 0.06*	0.70 ± 0.09** ^d
Seminal Vesicles with Coagulating Gland				
Absolute (g)	1.321 ± 0.034**	1.409 ± 0.083	1.154 ± 0.070	0.396 ± 0.067** ^d
Relative (mg/g)	3.32 ± 0.12**	3.43 ± 0.15	3.32 ± 0.19	1.47 ± 0.25** ^d
Right Testis				
Absolute (g)	1.961 ± 0.046**	1.971 ± 0.031	1.816 ± 0.040	1.402 ± 0.076**
Relative (mg/g)	4.92 ± 0.14*	4.85 ± 0.14	5.23 ± 0.11	5.30 ± 0.24
Left Testis				
Absolute (g)	1.920 ± 0.046**	1.944 ± 0.036	1.800 ± 0.037	1.415 ± 0.073**
Relative (mg/g)	4.82 ± 0.13**	4.77 ± 0.10	5.19 ± 0.08	5.35 ± 0.23*
Right Epididymis				
Absolute (g)	0.653 ± 0.009**	0.643 ± 0.022	0.607 ± 0.020	0.424 ± 0.033**
Relative (mg/g)	1.64 ± 0.04	1.57 ± 0.04	1.75 ± 0.05	1.60 ± 0.12
Left Epididymis				
Absolute (g)	0.643 ± 0.011**	0.639 ± 0.014	0.592 ± 0.014	0.432 ± 0.028**
Relative (mg/g)	1.61 ± 0.04	1.57 ± 0.04	1.71 ± 0.04	1.64 ± 0.10

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData are displayed as mean ± standard error of the litter means.

^bStatistical analysis performed using the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^cRelative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

^dn = 9 due to removal of outliers.

Bisphenol AF, NTP DART 08

Table 37. Summary of Organ Weights of Adult F₁ Male Rats in the Prenatal and Reproductive Performance Cohorts Exposed to Bisphenol AF in Feed^{a,b,c}

	0 ppm		338 ppm		1,125 ppm		3,750 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC
No. of Litters Examined	22	22	23	21	21	22	20	20
Necropsy Body Wt. (g)	451.7 ± 6.6**	419.1 ± 5.6**	421.9 ± 7.9**	402.8 ± 5.6	389.2 ± 5.8**	360.6 ± 7.0**	272.8 ± 6.5**	261.9 ± 5.9**
Dorsolateral Prostate Gland								
Absolute (g)	0.521 ± 0.029**	0.488 ± 0.028**	0.497 ± 0.018	0.441 ± 0.017	0.487 ± 0.015 ^d	0.411 ± 0.022*	0.169 ± 0.021**	0.168 ± 0.016*** ^e
Relative (mg/g) ^f	1.15 ± 0.06**	1.17 ± 0.07**	1.18 ± 0.04	1.09 ± 0.04	1.25 ± 0.04 ^d	1.15 ± 0.07	0.61 ± 0.07**	0.64 ± 0.05*** ^e
Ventral Prostate Gland								
Absolute (g)	0.714 ± 0.035**	0.627 ± 0.031**	0.714 ± 0.027	0.571 ± 0.026	0.616 ± 0.027*	0.466 ± 0.027**	0.172 ± 0.024**	0.161 ± 0.022*** ^d
Relative (mg/g)	1.59 ± 0.08**	1.50 ± 0.08**	1.70 ± 0.06	1.42 ± 0.06	1.59 ± 0.07	1.29 ± 0.07*	0.61 ± 0.07**	0.61 ± 0.08*** ^d
Seminal Vesicles with Coagulating Gland								
Absolute (g)	1.706 ± 0.044**	1.466 ± 0.055**	1.589 ± 0.043	1.384 ± 0.032	1.515 ± 0.058*	1.318 ± 0.053*	0.344 ± 0.069**	0.362 ± 0.058*** ^e
Relative (mg/g)	3.79 ± 0.10**	3.50 ± 0.13**	3.79 ± 0.12	3.44 ± 0.08	3.89 ± 0.13	3.68 ± 0.15	1.20 ± 0.22**	1.35 ± 0.19*** ^e
Levator Ani/bulbocavernosus Muscle Complex								
Absolute (g)	1.243 ± 0.027**	1.231 ± 0.036**	1.190 ± 0.024	1.152 ± 0.030	1.155 ± 0.022*	1.087 ± 0.026**	0.497 ± 0.040**	0.471 ± 0.044**
Relative (mg/g)	2.76 ± 0.07**	2.94 ± 0.09**	2.84 ± 0.07	2.86 ± 0.07	2.97 ± 0.06	3.03 ± 0.09	1.80 ± 0.12**	1.78 ± 0.14**
Cowper's Glands								
Absolute (g)	0.1198 ± 0.0024**	0.1121 ± 0.0040**	0.0990 ± 0.0044**	0.1047 ± 0.0038	0.1004 ± 0.0033**	0.0935 ± 0.0041*** ^g	0.0404 ± 0.0046*** ^g	0.0371 ± 0.0033*** ^h
Relative (mg/g)	0.27 ± 0.00**	0.27 ± 0.01**	0.23 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01 ^g	0.14 ± 0.01*** ^g	0.14 ± 0.01*** ^h
Right Testis								
Absolute (g)	1.997 ± 0.077**	1.928 ± 0.086**	1.978 ± 0.027	1.883 ± 0.028	1.884 ± 0.044	1.772 ± 0.046	1.396 ± 0.087**	1.340 ± 0.056**
Relative (mg/g)	4.43 ± 0.17**	4.58 ± 0.21**	4.71 ± 0.08	4.69 ± 0.08	4.84 ± 0.09	4.93 ± 0.13	5.08 ± 0.29*	5.16 ± 0.23*

Bisphenol AF, NTP DART 08

	0 ppm		338 ppm		1,125 ppm		3,750 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC
Left Testis								
Absolute (g)	1.965 ± 0.078**	2.021 ± 0.029**	1.965 ± 0.028	1.903 ± 0.030*	1.876 ± 0.047	1.776 ± 0.047**	1.469 ± 0.057**	1.355 ± 0.054**
Relative (mg/g)	4.36 ± 0.17**	4.85 ± 0.11**	4.68 ± 0.08	4.74 ± 0.08	4.82 ± 0.10*	4.95 ± 0.13	5.37 ± 0.16**	5.22 ± 0.22
Right Epididymis								
Absolute (g)	0.646 ± 0.020**	0.699 ± 0.023**	0.629 ± 0.009	0.666 ± 0.008	0.587 ± 0.014*	0.623 ± 0.013**	0.385 ± 0.027**	0.407 ± 0.022**
Relative (mg/g)	1.43 ± 0.04	1.66 ± 0.05	1.50 ± 0.02	1.66 ± 0.03	1.51 ± 0.03	1.74 ± 0.04	1.39 ± 0.09	1.55 ± 0.08
Left Epididymis								
Absolute (g)	0.656 ± 0.019**	0.708 ± 0.012**	0.648 ± 0.010	0.661 ± 0.009*	0.602 ± 0.013*	0.621 ± 0.011**	0.405 ± 0.020**	0.410 ± 0.019**
Relative (mg/g)	1.45 ± 0.04	1.69 ± 0.03	1.54 ± 0.02	1.65 ± 0.03	1.55 ± 0.03	1.73 ± 0.04	1.47 ± 0.06	1.57 ± 0.07
Preputial Glands								
Absolute (g)	0.1504 ± 0.0089**	0.1667 ± 0.0106**	0.1508 ± 0.0127	0.1527 ± 0.0154	0.1235 ± 0.0072	0.1407 ± 0.0107 ^f	0.1022 ± 0.0070**	0.1089 ± 0.0054**
Relative (mg/g)	0.33 ± 0.02	0.40 ± 0.03	0.36 ± 0.03	0.38 ± 0.04	0.32 ± 0.02	0.39 ± 0.03	0.38 ± 0.03	0.42 ± 0.02

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

RPC = reproductive performance cohort; PC = prenatal cohort.

^aData for the RPC and PC are also presented separately by cohort in Appendix E.

^bData are displayed as mean ± standard error of the litter means.

^cStatistical analysis performed using the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^dn = 20 due to removal of outliers.

^en = 19 due to removal of outliers.

^fRelative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

^gn = 21 due to removal of outliers.

^hn = 18 due to removal of outliers.

Female Necropsies

The F₁ females in the reproductive performance cohort were euthanized at 158–175 days of age when their F₂ pups reached PND 28. F₁ females in the prenatal cohort were euthanized on the assumed GD 21 of pregnancy with the F₂ generation at 123–137 days of age, and the F₁ subchronic cohort females were 116–120 days of age at the time of necropsy. There were BPAF-related gross findings, including three F₁ females in the 3,750 ppm group with malformations of the vagina: one had no apparent vaginal opening and two had a misshapen vagina (Table 38, Table 39). Terminal body (necropsy) weights of rats in the 1,125 and 3,750 ppm groups were significantly decreased by 11%–20% and 23%, respectively, relative to the terminal body weight of the control animals (Table 40, Table 41).

Absolute ovarian weights (left and right) were lower by 7%–14% for the 338 ppm group and significantly decreased by 17%–38% and 63%–64% below the control group for the 1,125 and 3,750 ppm groups, respectively, across the three cohorts (only the subchronic cohort was evaluated at 3,750 ppm) (Table 40, Table 41). Relative ovarian weights were significantly decreased in the 1,125 ppm group (the lower weights of the right ovary in the subchronic cohort and the left and right ovaries in the prenatal cohort were not significant) and in the 3,750 ppm group. The magnitude of the reduction in weights of the ovaries for the 1,125 and 3,750 ppm groups was more than the magnitude of the reduction in body weight relative to the control group, suggesting a direct BPAF-mediated suppression of maturation of this tissue.

The uterus was weighed with the cervix and vagina intact in the subchronic cohort so that it could be processed appropriately for histopathological examination (Table 40). The absolute weight of the uterus/cervix/vagina was lower by 16% for the 338 ppm group and significantly decreased by 31% and 37% for the 1,125 and 3,750 ppm groups, respectively, relative to the control group. Relative weights were lower in all three exposed groups but were not statistically different from the control group. The magnitude of the reduction in weight of the uterus/cervix/vagina was more than the magnitude of the reduction in body weight, suggesting a direct BPAF-mediated suppression of maturation of this tissue.

Absolute kidney (left and right), lung (only at 3,750 ppm), and heart weights were significantly decreased by 9%–16% and 19%–21% below the control group for the 1,125 and 3,750 ppm groups in the subchronic cohort, respectively (Table 40). Relative kidney, lung, and heart weights were not significantly different from the respective control groups, suggesting that the changes in absolute weights of those organs might have been secondary to the effect of BPAF on body weight. There was no effect of BPAF exposure on kidney (left and right), lung, and heart weights for the 338 ppm group.

Absolute weights of the adrenal glands were lower by 10% and 12% for the 338 and 1,125 ppm groups in the subchronic cohort, respectively, and significantly decreased to 26% below the control group for the 3,750 ppm group (Table 40). Relative adrenal gland weights were not significantly different from the control group, suggesting that the changes in absolute weights might have been secondary to the effect of BPAF on body weight.

Absolute weights of the thyroid were not significantly different from the respective control groups in the subchronic cohort (Table 40). Absolute liver and thymus weights showed a negative trend with exposure concentration. Relative thyroid and liver weights were significantly increased from the control group, indicating that the thyroid and liver were large relative to the

size of the animals. Relative thymus weights were not significantly different from the control group, suggesting that the changes in absolute weights of the thymus might have been secondary to the effect of BPAF on body weight. There was no effect of BPAF exposure on thyroid, liver, or thymus weights for the 338 and 1,125 ppm groups.

Table 38. Summary of Gross Necropsy Findings in Adult F₁ Female Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed^a

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. of Animals Examined ^b	10 (10)	10 (10)	10 (10)	10 (10)
Ovaries				
Reduced				
Bilateral ^c	0**	0	0	9 (9)**
Uterus				
Reduced				
Bilateral	0**	0	0	9 (9)**

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

^aStatistical analysis performed using the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.

^bNumber of animals (number of litters) examined for gross lesions.

^cNumber of animals (number of litters) with lesion.

Table 39. Summary of Gross Necropsy Findings in Adult F₁ Female Rats in the Prenatal and Reproductive Performance Cohorts Exposed to Bisphenol AF in Feed^{a,b}

	0 ppm		338 ppm		1,125 ppm		3,750 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC
No. of Animals Examined ^c	22 (22)	22 (22)	24 (24)	21 (21)	21 (21)	22 (22)	20 (20)	20 (20)
Ovaries								
Reduced								
Left ^d	0	0*	0	0	0	0	0	2 (2)
Right	0	0*	0	0	0	0	0	2 (2)
Bilateral	0**	0**	0	0	0	1 (1)	18 (18)**	17 (17)**
Total	0**	0**	0	0	0	1 (1)	18 (18)**	19 (19)**
Uterus								
Reduced								
Bilateral	0	0**	0	0	0	1 (1)	0	19 (19)**
Vagina								
Deformity	0	0	0	0	0	0	1 (1)	0
Misshapen	0	0	0	0	0	0	1 (1)	1 (1)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

RPC = reproductive performance cohort; PC = prenatal cohort.

^aData for the RPC and PC are also presented separately by cohort in Appendix E.

^bStatistical analysis performed using the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.

^cNumber of animals (number of litters) examined for gross lesions.

^dNumber of animals (number of litters) with lesion.

Table 40. Summary of Organ Weights of Adult F₁ Female Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed^{a,b}

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. of Litters Examined	10	10	10	10
Necropsy Body Wt. (g)	253.1 ± 5.7**	234.9 ± 9.9	214.8 ± 3.3**	194.1 ± 7.8**
Right Ovary				
Absolute (g)	0.0766 ± 0.0024**	0.0716 ± 0.0064	0.0633 ± 0.0048*	0.0283 ± 0.0015**
Relative (mg/g) ^c	0.30 ± 0.01**	0.30 ± 0.02	0.29 ± 0.02	0.15 ± 0.01**
Left Ovary				
Absolute (g)	0.0812 ± 0.0032**	0.0743 ± 0.0049	0.0593 ± 0.0031**	0.0289 ± 0.0031**
Relative (mg/g)	0.32 ± 0.01**	0.32 ± 0.02	0.28 ± 0.01*	0.15 ± 0.01**
Uterus, Cervix, and Vagina				
Absolute (g)	1.002 ± 0.079**	0.843 ± 0.127	0.694 ± 0.054*	0.632 ± 0.091**
Relative (mg/g)	4.00 ± 0.35	3.70 ± 0.62	3.21 ± 0.21	3.25 ± 0.43
Right Kidney				
Absolute (g)	0.89 ± 0.02**	0.84 ± 0.04	0.75 ± 0.03**	0.71 ± 0.04**
Relative (mg/g)	3.54 ± 0.05	3.57 ± 0.06	3.48 ± 0.10	3.71 ± 0.20
Left Kidney				
Absolute (g)	0.87 ± 0.01**	0.83 ± 0.04	0.76 ± 0.03**	0.69 ± 0.03**
Relative (mg/g)	3.46 ± 0.06	3.55 ± 0.05	3.53 ± 0.10	3.58 ± 0.15
Lung				
Absolute (g)	1.72 ± 0.06**	1.68 ± 0.09	1.57 ± 0.06	1.37 ± 0.08**
Relative (mg/g)	6.78 ± 0.18	7.15 ± 0.28	7.32 ± 0.22	7.14 ± 0.41
Heart				
Absolute (g)	1.01 ± 0.03**	1.03 ± 0.03	0.92 ± 0.02*	0.82 ± 0.03**
Relative (mg/g)	4.02 ± 0.08	4.40 ± 0.09	4.28 ± 0.12	4.26 ± 0.16
Adrenal Glands				
Absolute (g)	0.0786 ± 0.0036**	0.0709 ± 0.0038	0.0691 ± 0.0027	0.0578 ± 0.0029**
Relative (mg/g)	0.31 ± 0.01	0.30 ± 0.01	0.32 ± 0.01	0.30 ± 0.02
Thyroid Gland				
Absolute (g)	0.0157 ± 0.0006	0.0161 ± 0.0008	0.0154 ± 0.0008	0.0145 ± 0.0008
Relative (mg/g)	0.06 ± 0.00**	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00*
Liver				
Absolute (g)	8.85 ± 0.25**	9.04 ± 0.51	8.34 ± 0.29	7.64 ± 0.38
Relative (mg/g)	35.00 ± 0.78*	38.38 ± 1.13	38.77 ± 0.94	39.56 ± 1.85*
Thymus				
Absolute (g)	0.278 ± 0.014**	0.293 ± 0.023	0.254 ± 0.019	0.227 ± 0.012
Relative (mg/g)	1.10 ± 0.05	1.24 ± 0.07	1.18 ± 0.08	1.17 ± 0.05

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData are displayed as mean ± standard error of the litter means.

^bStatistical analysis performed using the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^cRelative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Bisphenol AF, NTP DART 08

Table 41. Summary of Ovary Weights of Adult F₁ Female Rats in the Prenatal and Reproductive Performance Cohorts Exposed to Bisphenol AF in Feed^{a,b,c}

	0 ppm		338 ppm		1,125 ppm		3,750 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC
No. of Litters Examined	18	16	20	20	9	15	– ^d	–
Necropsy Body Wt. (g) ^e	306.5 ± 4.0**	311.7 ± 3.3**	284.6 ± 3.5**	282.8 ± 4.4**	272.2 ± 5.3**	249.5 ± 5.3**	–	–
Right Ovary								
Absolute (g)	0.0748 ± 0.0038**	0.0848 ± 0.0067**	0.0644 ± 0.0043	0.0782 ± 0.0046	0.0464 ± 0.0053**	0.0567 ± 0.0048**	–	–
Relative (mg/g) ^f	0.24 ± 0.01*	0.27 ± 0.02	0.23 ± 0.01	0.28 ± 0.02	0.17 ± 0.02**	0.23 ± 0.02	–	–
Left Ovary								
Absolute (g)	0.0745 ± 0.0038**	0.0825 ± 0.0042**	0.0638 ± 0.0044	0.0717 ± 0.0043	0.0467 ± 0.0061**	0.0539 ± 0.0041**	–	–
Relative (mg/g)	0.24 ± 0.01*	0.26 ± 0.01*	0.22 ± 0.01	0.25 ± 0.02	0.17 ± 0.02**	0.22 ± 0.02	–	–

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

RPC = reproductive performance cohort; PC = prenatal cohort.

^aData for the RPC and PC are also presented separately by cohort in Appendix E.

^bData are displayed as mean ± standard error of the litter means.

^cStatistical analysis performed using the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

^dNone of the females in the 3,750 ppm group were sperm-positive, so no organ weight data were collected as the females were terminated at the end of cohabitation.

^eThe terminal body weight for the prenatal females is the final body weight minus the gravid uterine weight.

^fRelative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Clinical Pathology

For male rats in the subchronic cohort, measured mean cell volume (MCV) and mean cell hemoglobin (MCH) displayed a mild but significant increase (5%) in the 3,750 ppm group. The reticulocyte count exhibited a positive trend with exposure concentration (Appendix E). The significantly increased MCV and MCH were likely due to the higher reticulocyte count compared to the control group. The higher number of reticulocytes might have resulted from biological variability or a redistribution of the circulating reticulocytes.

For the 3,750 ppm females, the hemoglobin concentration and erythrocyte count displayed a mild but significant decrease ($\leq 6\%$) compared to the control group. In addition, the white blood cell count was significantly decreased (26%) in the 3,750 ppm animals, and the monocyte and basophils counts were significantly decreased in most exposed groups relative to the control group. While there was no significant pairwise comparison, there was a negative trend in the lymphocyte count with exposure concentration (Table 42).

Table 42. Summary of Select Hematology Data for F₁ Adult Female Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed^{a,b}

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
n	10	10	10	10
Erythrocytes (10 ⁶ /μL)	8.81 ± 0.15**	8.68 ± 0.07	8.55 ± 0.10	8.27 ± 0.09**
Hemoglobin (g/dL)	16.1 ± 0.2**	15.9 ± 0.1	15.7 ± 0.2	15.3 ± 0.2**
Mean Cell Hemoglobin Concentration (g/dL)	31.6 ± 0.2	31.4 ± 0.1	31.4 ± 0.1	31.3 ± 0.1
Mean Cell Volume (fL)	57.9 ± 0.4*	58.3 ± 0.4	58.5 ± 0.6	59.2 ± 0.3
Reticulocytes (10 ³ /μL)	206.3 ± 12.7	214.4 ± 11.4	210.0 ± 12.9	236.3 ± 17.7
White Blood Cells (10 ³ /μL)	10.36 ± 0.72**	9.57 ± 0.92	8.57 ± 0.64	7.70 ± 0.80*
Neutrophils (10 ³ /μL)	1.42 ± 0.16	1.16 ± 0.09	1.05 ± 0.11	1.17 ± 0.19
Lymphocytes (10 ³ /μL)	7.76 ± 0.54*	7.48 ± 0.84	6.83 ± 0.52	5.81 ± 0.66
Monocytes (10 ³ /μL)	0.39 ± 0.04**	0.33 ± 0.04	0.27 ± 0.03*	0.26 ± 0.05*
Basophils (10 ³ /μL)	0.25 ± 0.03**	0.14 ± 0.02**	0.12 ± 0.01**	0.10 ± 0.02**

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData displayed as mean ± standard error.

^bStatistical analysis performed using the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

Several significant changes were observed in the clinical chemistry parameters (Table 43). Cholesterol concentrations were significantly decreased in the 1,125 and 3,750 ppm male rat groups and in all the BPAF-exposed female groups, relative to the respective control groups. In the 3,750 ppm females, triglyceride concentrations were significantly increased relative to the control group. In male rats, bile acid concentrations were significantly decreased in the 1,125 and 3,750 ppm groups, with the 3,750 ppm group being only 18% of the control group. In male rats, globulin concentrations were minimally but significantly decreased, which drove a mild significant decrease in the total protein concentration. Conversely, the 3,750 ppm female rats exhibited significantly increased globulin concentrations that resulted in a significant decrease in

the albumin/globulin ratio relative to the control group. The relevance of these disparate mild globulin changes is uncertain.

Table 43. Summary of Select Clinical Chemistry Data for F₁ Male and Female Adult Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed^{a,b}

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Male				
n	10	10	10	9
Total Protein (g/dL)	6.80 ± 0.06*	6.62 ± 0.08	6.60 ± 0.08	6.56 ± 0.06*
Albumin (g/dL)	3.41 ± 0.03	3.33 ± 0.04	3.36 ± 0.05	3.36 ± 0.03
Globulin (g/dL)	3.39 ± 0.04**	3.29 ± 0.05	3.24 ± 0.05*	3.20 ± 0.05**
A/G Ratio	1.01 ± 0.01*	1.01 ± 0.01	1.04 ± 0.01	1.05 ± 0.02
Cholesterol (mg/dL)	94.3 ± 4.0**	87.7 ± 4.5	75.2 ± 3.2**	58.8 ± 4.1**
Triglycerides (mg/dL)	154.7 ± 12.7	177.2 ± 13.9	172.8 ± 14.0	191.1 ± 15.2
Bile Acids (µmol/L)	32.7 ± 4.4**	25.3 ± 3.8	19.7 ± 2.8*	6.0 ± 0.3**
Female				
n	10	10	10	10
Total Protein (g/dL)	6.50 ± 0.10	6.32 ± 0.11	6.41 ± 0.09	6.65 ± 0.11
Albumin (g/dL)	3.48 ± 0.05	3.31 ± 0.04	3.31 ± 0.05	3.34 ± 0.05
Globulin (g/dL)	3.02 ± 0.06**	3.01 ± 0.07	3.10 ± 0.06	3.31 ± 0.07**
A/G Ratio	1.15 ± 0.02**	1.10 ± 0.02	1.07 ± 0.02**	1.01 ± 0.02**
Cholesterol (mg/dL)	100.1 ± 2.4**	87.5 ± 3.6*	74.5 ± 5.7**	63.2 ± 4.8**
Triglycerides (mg/dL)	101.4 ± 11.6*	108.0 ± 11.3	119.2 ± 20.2	167.8 ± 21.3*
Bile Acids (µmol/L)	23.3 ± 6.2	25.9 ± 5.5	23.4 ± 3.2	10.5 ± 1.8

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

A/G Ratio = albumin/globulin ratio.

^aData are presented as mean ± standard error.

^bStatistical analysis performed using the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

Histopathology

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions in male and female reproductive organs in the reproductive performance and subchronic cohorts and nonneoplastic lesions in the kidneys in the subchronic cohort. Summaries of the incidences of nonneoplastic lesions are presented in Table 44 and Table 45 for male reproductive performance and subchronic cohort rats, respectively, in Table 46 and Table 47 for female reproductive performance and subchronic cohort rats, respectively, and are also presented as supplemental data in Appendix E.

Testes: There was a significant increase in the incidence of germinal epithelium degeneration in the 3,750 ppm reproductive performance cohort and a positive trend in the subchronic cohort with exposure concentration (Table 44, Table 45). The incidences of Leydig cell atrophy and

seminiferous tubule spermatid retention were significantly increased in the 3,750 ppm reproductive performance cohort but not in males exposed to lower concentrations, when compared to the control group. Degeneration in the testes encompassed several changes, including vacuolation of germinal epithelium, reduction and focal loss of elongating spermatids, and disorganization of germ cell layers (Figure 34). The severity was generally noted as minimal and was often accompanied by exfoliated germ cells in profiles of the epididymal duct lumen. Leydig cell atrophy was characterized by decreased number and size of Leydig cells (Figure 35). Seminiferous tubule spermatid retention was characterized by persistence of the most mature elongating spermatids after the stage of physiological release, which occurs at stage VIII. Mature elongating spermatids were present at or near the luminal surface in stage IX–XI testes.

Table 44. Incidences of Select Nonneoplastic Lesions in Adult F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed^a

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. Examined ^b	22 (22)	24 (24)	21 (21)	20 (20)
Testis				
Germinal epithelium, degeneration ^c	0**	0	1 (1) [2.0] ^d	6 (6)** [1.3]
Leydig cell, atrophy	0**	0	0	11 (11)** [1.8]
Seminiferous tubule, retention, spermatid	0**	0	0	8 (8)** [1.0]
Epididymis				
Duct, atrophy	0**	0	0	10 (10)** [1.4]
Duct, hypospermia	0**	0	1 (1) [1.0]	6 (6)** [2.0]
Duct, exfoliated germ cell	0**	0	1 (1) [1.0]	5 (5)* [1.4]
Prostate Gland				
Hypoplasia, dorsolateral	0**	0	0	18 (18)** [2.6]
Hypoplasia, ventral	0**	0	0	18 (18)** [2.6]
Seminal Vesicle				
Hypoplasia, bilateral	0**	0	0	18 (18)** [2.6]
Coagulating Gland				
Hypoplasia, bilateral	0**	0	0	18 (18)** [2.6]
Cowper's Glands				
Hypoplasia, bilateral	0**	0	0	15 (15)** [2.3] ^e
Hypoplasia, unilateral	0	1 (1) [4.0]	0	0
Hypoplasia, total	0**	1 (1) [4.0]	0	15 (15)** [2.3]
Levator Ani/bulbocavernosus Muscle Complex				
Hypoplasia	0**	0	1 (1) [2.0]	17 (17)** [2.4]

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aStatistical analysis performed using the Cochran-Armitage test with a Poly-3 adjustment for both trend and pairwise tests.

^bNumber of animals (number of litters) with tissue examined microscopically.

^cNumber of animals (number of litters) with lesion.

^dAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

^eTwo animals in the 3,750 ppm group were not examined for this lesion.

Table 45. Incidences of Select Nonneoplastic Lesions in Adult F₁ Male Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed^a

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. Examined ^b	10 (10)	10 (10)	10 (10)	10 (10)
Testis				
Germinal epithelium, degeneration ^c	0*	0	0	2 (2) [1.5] ^d
Epididymis				
Duct, exfoliated germ cell	0**	0	0	3 (3) [1.7]
Prostate Gland				
Hypoplasia, dorsolateral	0**	0	0	10 (10)** [1.5]
Hypoplasia, ventral	0**	0	0	10 (10)** [1.5]
Seminal Vesicle				
Hypoplasia, bilateral	0**	0	0	10 (10)** [1.6]
Kidney				
Corticomedullary junction, mineral	0	— ^e	—	7 (7)** [1.1]

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aStatistical analysis performed using the Cochran-Armitage test with a Poly-3 adjustment for both trend and pairwise tests.

^bNumber of animals (number of litters) with tissue examined microscopically.

^cNumber of animals (number of litters) with lesion.

^dAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

^eNo animals evaluated at 338 and 1,125 ppm.

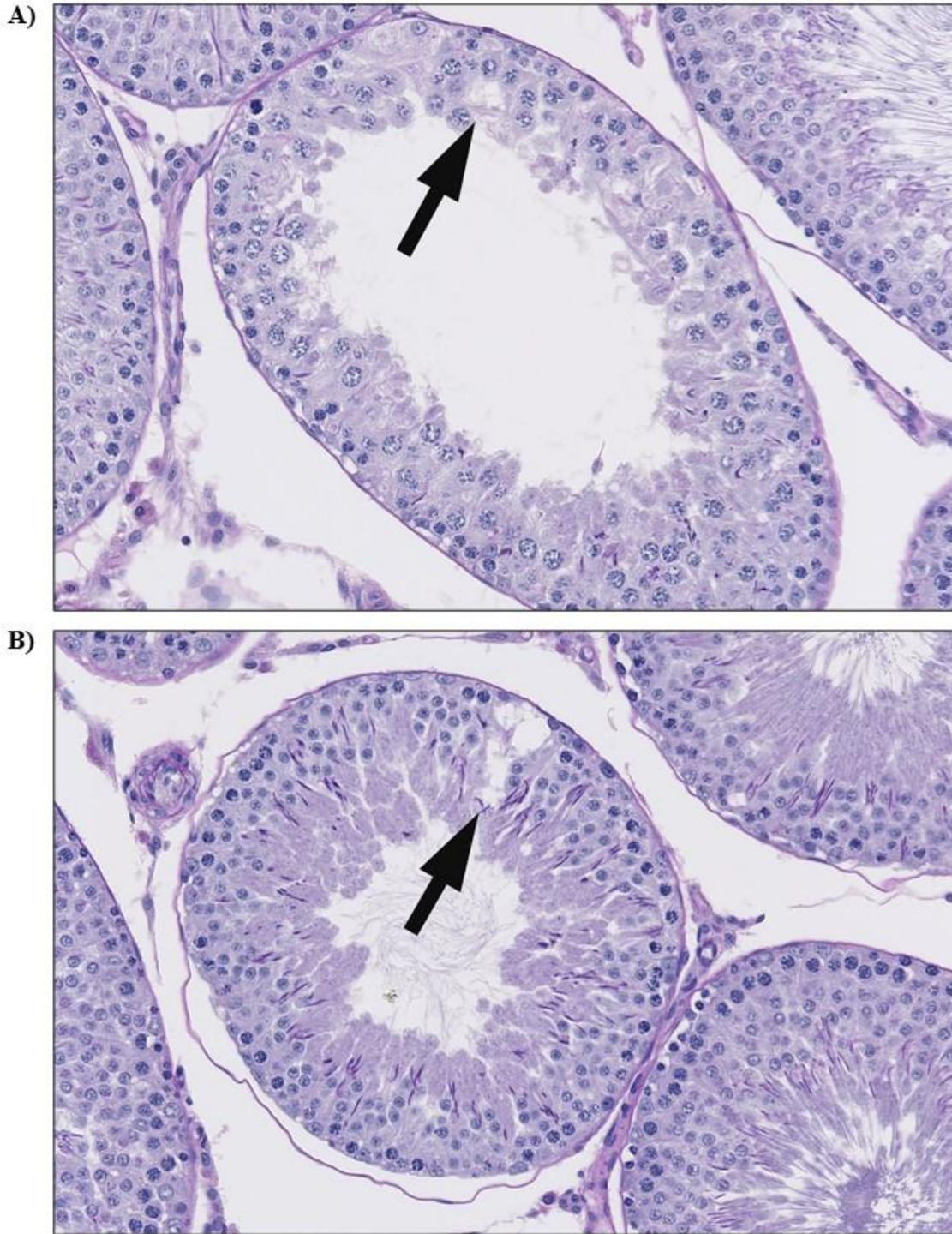


Figure 34. Representative Images of Germinal Epithelial Degeneration in the Testis of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

(A) An example of germinal epithelial degeneration is shown from a 3,750 ppm reproductive performance cohort male; this is a late stage tubule with general depletion of the elongating spermatids and disorganization of the pachytene spermatocytes in the germinal epithelium, as well as focal areas of germ cell drop out (arrow; 20x). (B) Another example of germinal epithelium degeneration is shown from a 3,750 ppm reproductive performance cohort male, where the main lesion of degeneration was vacuolation (arrow; 20x). H&E = hematoxylin and eosin stain.

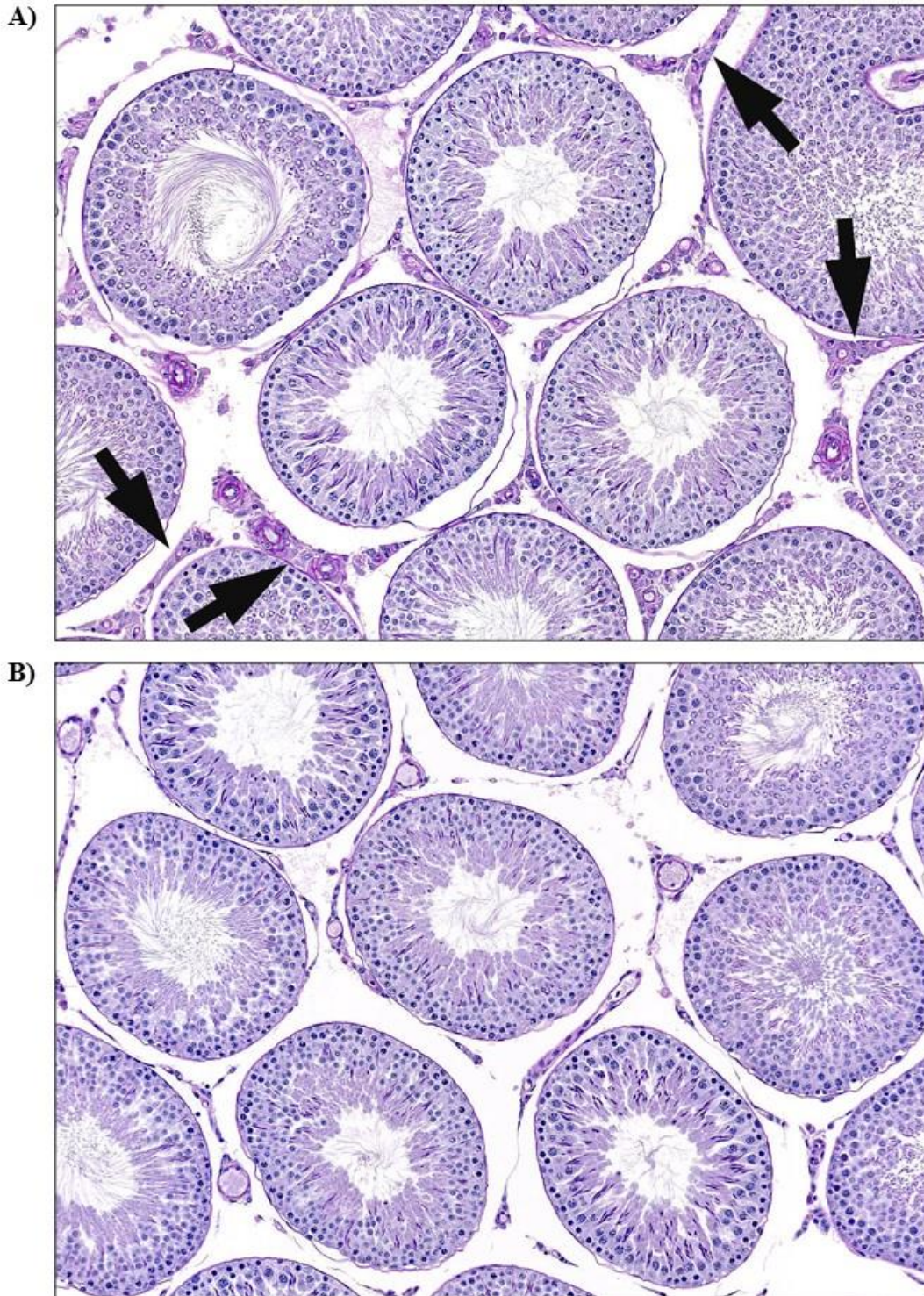


Figure 35. Representative Images of Leydig Cell Atrophy in the Testis of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

(A) Leydig (interstitial) cells next to the seminiferous tubules (arrows) are shown in a control reproductive performance cohort male (10x). (B) Atrophied Leydig cells are shown from a 3,750 ppm reproductive performance cohort male (10x). There is a decrease in both the size and number of Leydig cells in panel B. The increased interstitial space in both panels is due to fixation artifact. H&E = hematoxylin and eosin stain.

Epididymis: There was a significant increase in the incidences of epididymis duct atrophy and duct hypospermia in the 3,750 ppm reproductive performance cohort relative to its control group (Table 44). There was a significant increase in epididymis duct exfoliated germ cell in the 3,750 ppm reproductive performance cohort and a positive trend in the subchronic cohort with exposure concentration (Table 44, Table 45). Epididymis duct atrophy was characterized by generalized or segmental decreased diameters of the duct lumens and increased interstitial stroma (Figure 36). Epididymis duct hypospermia was characterized by a reduced density of sperm in the epididymal duct lumen. (Figure 36). Exfoliated germ cells consisted of numerous individualized sloughed germinal epithelial cells, often with condensed nuclei, and debris within the epididymal duct profiles (Figure 37).

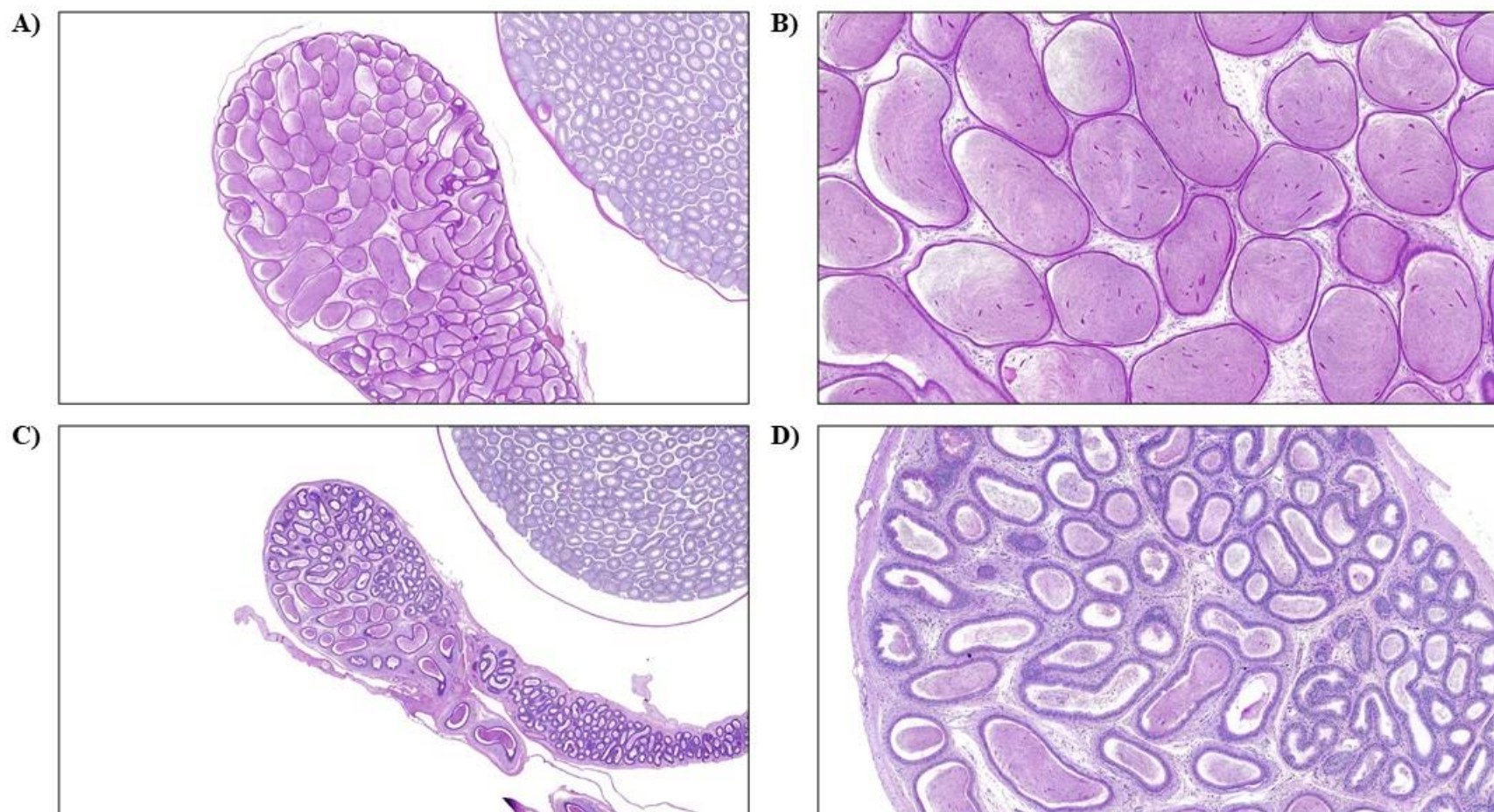


Figure 36. Representative Images of Duct Atrophy and Hypospermia in the Epididymis of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

(A) Normal size and histological appearance of duct profiles in the cauda epididymis are shown from a control reproductive performance cohort male rat (1.25x). (B) The duct profiles in the cauda epididymis are shown from a control reproductive performance cohort male rat (5x). (C) Epididymis duct atrophy with hypospermia is shown from a 3,750 ppm reproductive performance cohort male (1.25x). (D) The epididymis duct atrophy with hypospermia is shown from a 3,750 ppm reproductive performance cohort male (5x). Epididymis duct atrophy resulted in an overall smaller epididymis size with decreased diameters of the duct profiles, intraductal infolding of the epithelium to form a scalloped appearance, and increased interstitial stroma. Hypospermia was frequently a concurrent lesion. A section of testis is in the upper right-hand corner of panels A and C. H&E = hematoxylin and eosin stain.

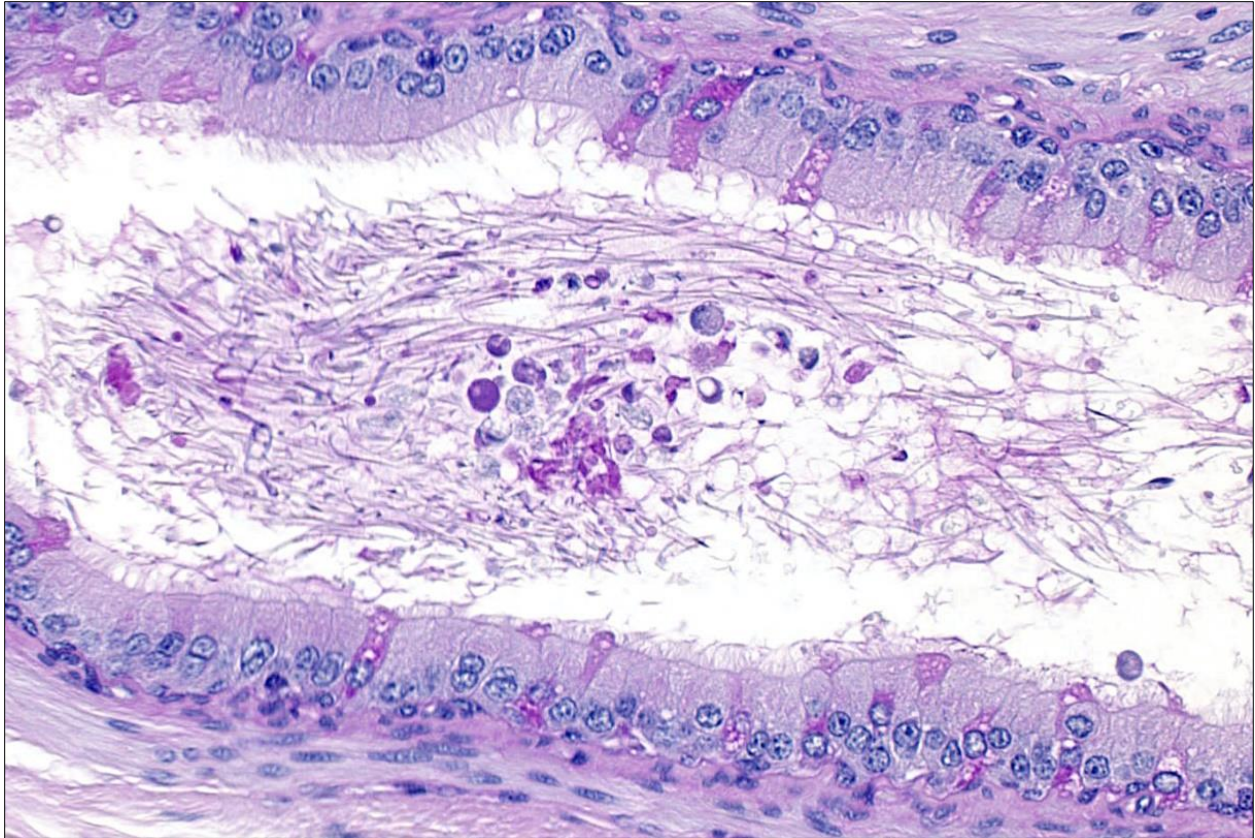


Figure 37. Representative Image of Exfoliated Germ Cells in the Ducts of the Epididymis of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

An example of a duct from the cauda epididymis with exfoliated germ cells is shown from a 3,750 ppm reproductive performance cohort male in Figure 36D (40x). Numerous individualized sloughed germinal epithelial cells are visible, often with condensed nuclei. H&E = hematoxylin and eosin stain.

Prostate gland: There were significant increases in the incidences of both dorsolateral and ventral prostate gland hypoplasia in the 3,750 ppm reproductive performance and subchronic cohorts (Table 44, Table 45). The prostate gland consists of a paired ventral portion and a paired dorsolateral portion, which together encircle the urethra. Hypoplasia was characterized by smaller glands with occasional malformed lobes compared to the control group (Figure 38).

Seminal vesicle: There was a significant increase in the incidences of bilateral hypoplasia in the 3,750 ppm reproductive performance and subchronic cohorts (Table 44, Table 45). Hypoplasia was characterized by smaller glands compared to the control group (Figure 38).

Coagulating and Cowper's glands: There was a significant increase in the incidences of bilateral hypoplasia of these glands in the 3,750 ppm reproductive performance cohort (Table 44). Hypoplasia was characterized by smaller glands compared to the control group (Figure 38).

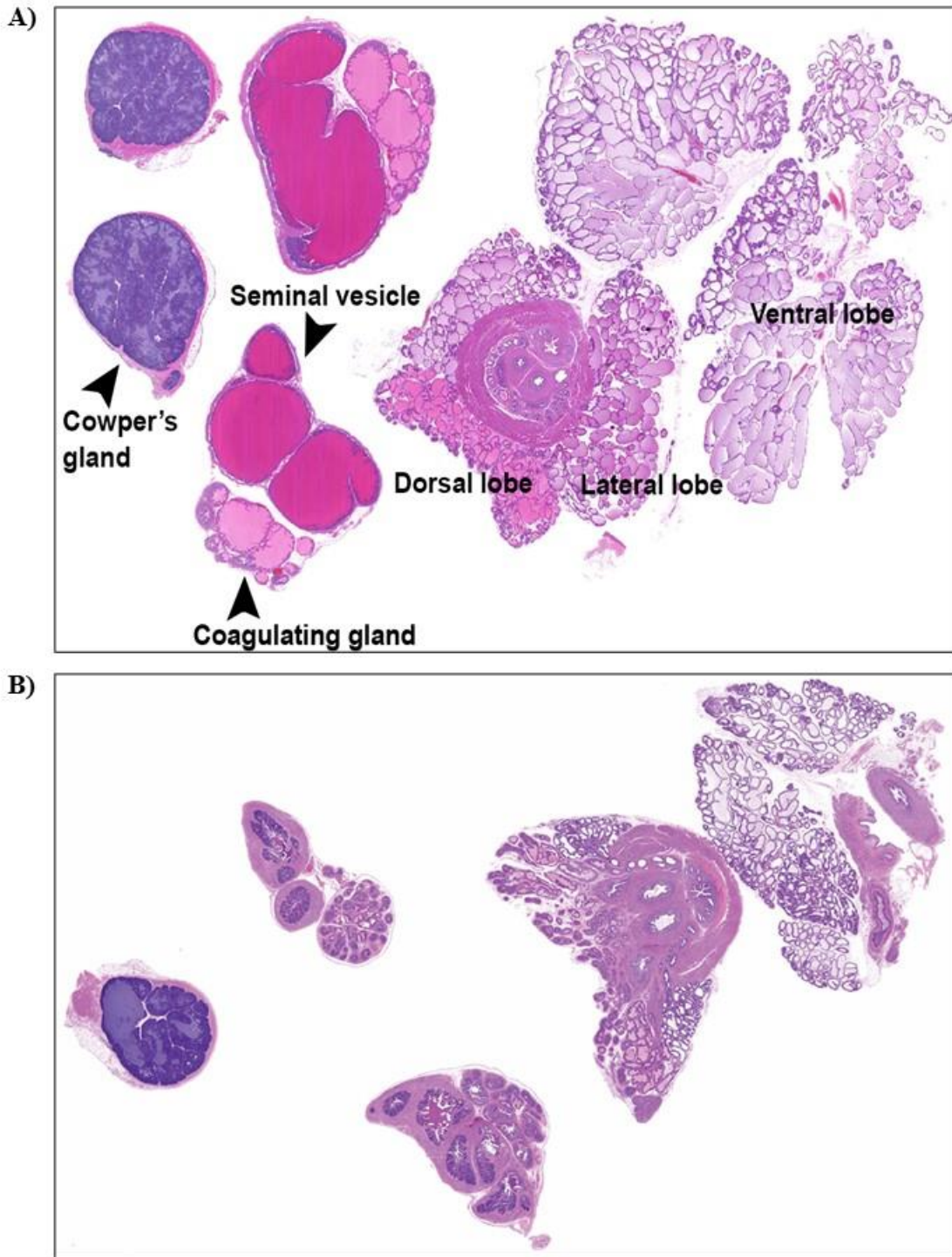


Figure 38. Representative Images of Hypoplasia in the Prostate Gland, Seminal Vesicle, Coagulating Gland, and Cowper's Gland of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

(A) Normal prostate (dorsal, ventral, and lateral lobes) gland, seminal vesicle, coagulating gland, and Cowper's gland are shown from a control reproductive performance cohort male (0.3x). (B) Hypoplasia of the corresponding tissues is shown from a 3,750 ppm reproductive performance cohort male at (0.6x). Hypoplasia was characterized by smaller tissues compared to the control group. H&E = hematoxylin and eosin stain.

Levator ani/bulbocavernosus (LABC) muscle complex: There was a significant increase in the incidence of LABC hypoplasia in the 3,750 ppm reproductive performance cohort (Table 44). Hypoplasia of these pelvic floor muscles was characterized by smaller muscles compared to the control group (Figure 39).

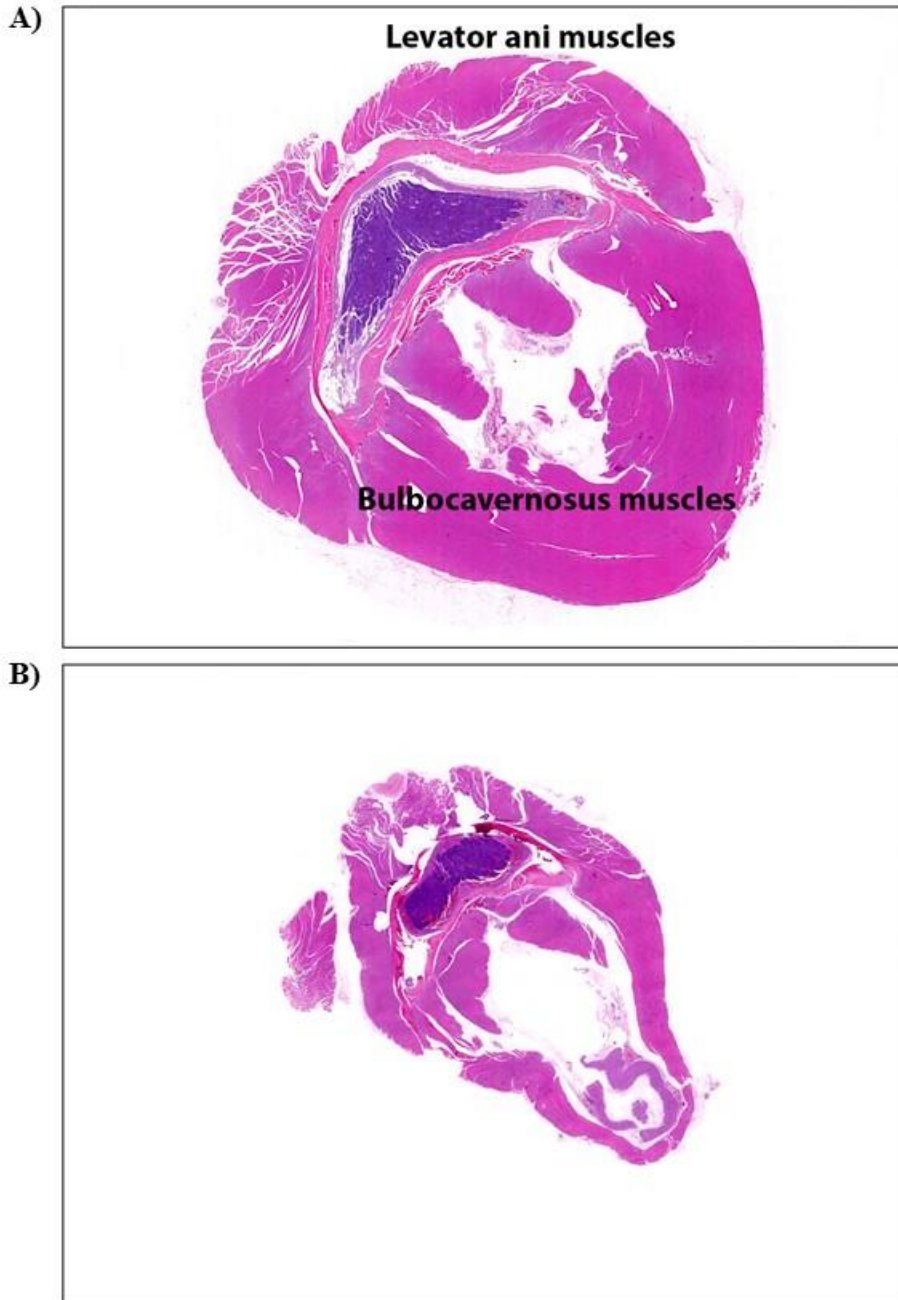


Figure 39. Representative Images of Hypoplasia in the Levator Ani/bulbocavernosus (LABC) Muscle Complex of F₁ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

(A) Normal LABC muscles are shown from a control reproductive performance cohort male (0.32x). (B) Hypoplastic LABC is shown from a 3,750 ppm reproductive performance cohort male (0.32x). Hypoplasia of this tissue was characterized by an overall smaller size but with normal architecture. H&E = hematoxylin and eosin stain.

Kidney: There was a significant increase in the incidence of mineral in the 3,750 ppm subchronic cohort (Table 45). This lesion consisted of focal, scattered deposits of dark basophilic granular material (mineral) noted primarily along the junction of the cortex and medulla (in the pars recta and thin loops of Henle near the junction of the outer and inner stripes of the outer medulla).

Ovary: There was a significant increase in the incidences of bilateral ovarian hypoplasia in the 3,750 ppm reproductive performance and subchronic cohorts (Table 46, Table 47). This lesion was characterized by an overall reduction in the size of the ovary accompanied by a reduction in numbers of corpora lutea, follicle maturation arrest (many secondary follicles present), and increased interstitial tissue (Figure 40). Primary follicles were present in the ovarian sections.

Table 46. Incidences of Select Nonneoplastic Lesions in Adult F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed^a

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. Examined ^b	22 (22)	24 (24)	21 (21)	20 (20)
Ovary				
Hypoplasia, bilateral ^c	0**	1 (1) [1.0] ^d	0	20 (20)** [2.6]
Hypoplasia, unilateral	0	2 (2) [1.0]	0	0
Hypoplasia, total	0**	3 (3) [1.0]	0	20 (20)** [2.6]
Uterus				
Hypoplasia	0**	0	0	18 (18)** [1.4]
Epithelial, metaplasia, squamous	0**	0	0	20 (20)** [1.0]
Dilation, glandular, cystic	0**	0	0	8 (8)** [1.1]
Stroma, hyalinization	0**	0	8 (8)** [1.4]	18 (18)** [3.0]
Epithelium, apoptosis, increased	0*	0	1 (1)	3 (3) ^e

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aStatistical analysis performed using the Cochran-Armitage test with a Poly-3 adjustment for both trend and pairwise tests.

^bNumber of animals (number of litters) with tissue examined microscopically.

^cNumber of animals (number of litters) with lesion.

^dAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

^eNo severity grade was used for the evaluation of this lesion, as directed by the Pathology Working Group.

Table 47. Incidences of Select Nonneoplastic Lesions in Adult F₁ Female Rats in the Subchronic Cohort Exposed to Bisphenol AF in Feed^a

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. Examined ^b	10 (10)	10 (10)	10 (10)	10 (10)
Ovary				
Hypoplasia, bilateral ^c	0**	0	0	10 (10)** [2.2] ^d
Uterus				
Hypoplasia	0**	0	0	10 (10)** [1.4]
Epithelial, metaplasia, squamous	0**	0	0	10 (10)** [1.3]
Dilation, glandular, cystic	0**	0	0	6 (6)** [1.8]
Stroma, hyalinization	0**	0	0	10 (10)** [3.0]

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

^aStatistical analysis performed using the Cochran-Armitage test with a Poly-3 adjustment for both trend and pairwise tests.

^bNumber of animals (number of litters) with tissue examined microscopically.

^cNumber of animals (number of litters) with lesion.

^dAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

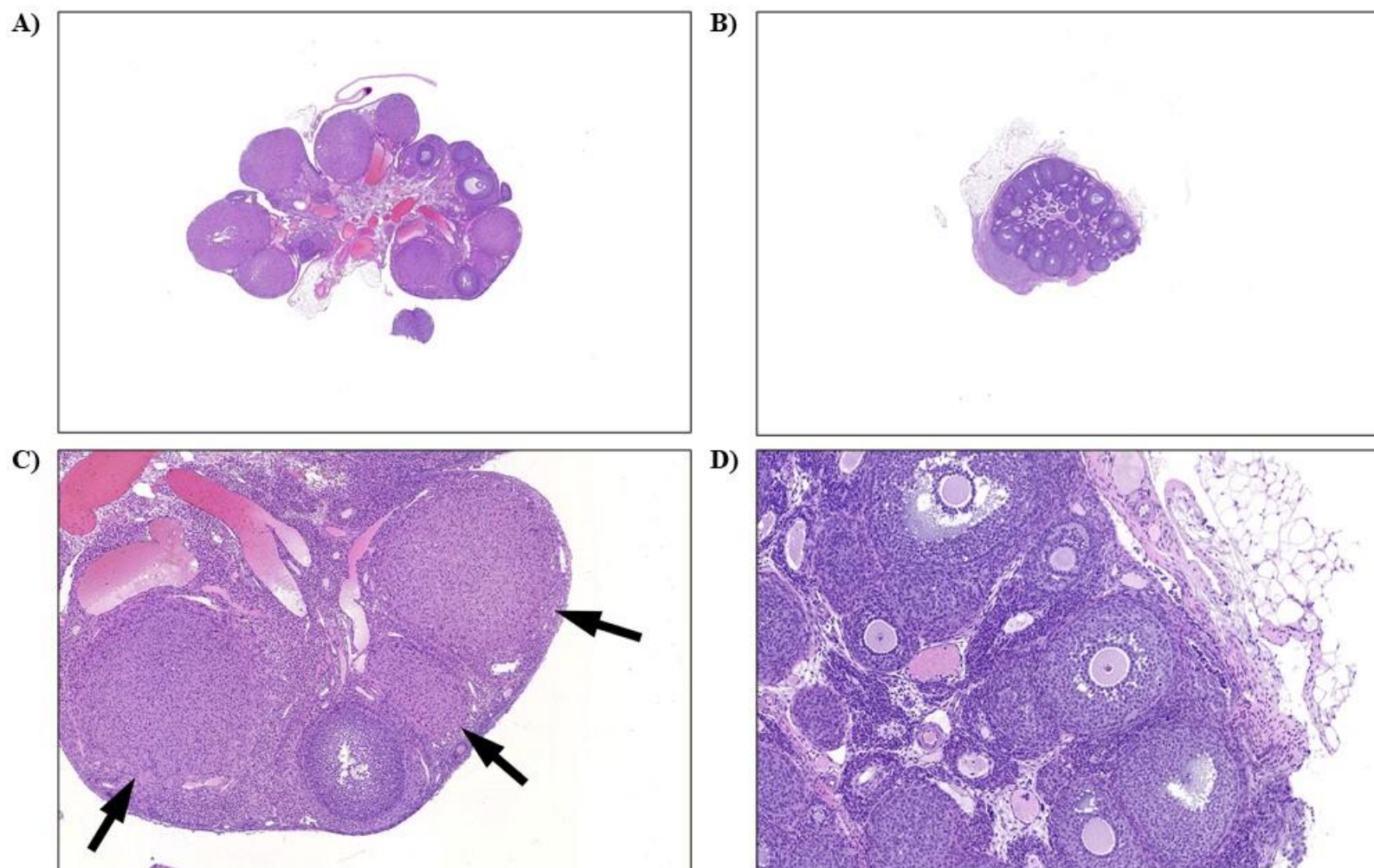


Figure 40. Representative Images of Hypoplasia in the Ovary of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

(A) Normal size and histological appearance of an ovary is shown from a control reproductive performance cohort female (1.25x). (B) An ovary is shown from a 3,750 ppm reproductive performance cohort female diagnosed with ovarian hypoplasia (1.16x). This lesion was characterized by an overall reduction in the size of the ovary due to a lack of, or reduction in, numbers of corpora lutea and reduced numbers of antral and/or growing follicles. (C) Higher magnification of panel A is shown (5x). Note the prominent corpora lutea (arrows). (D) Higher magnification of panel B is shown (10x). H&E = hematoxylin and eosin stain.

Uterus: There were significant increases in the incidences of hypoplasia, epithelial squamous metaplasia, and cystic glandular dilation in the 3,750 ppm reproductive performance and subchronic cohorts (Table 46, Table 47). There were significant increases in stromal hyalinization in the 1,125 and 3,750 ppm reproductive performance cohorts and in the 3,750 ppm subchronic cohort. There was also a positive trend for uterine epithelium apoptosis in the reproductive performance cohort with exposure concentration. Apoptosis of the uterine luminal epithelium is a normal physiologic response during the estrous cycle. Uterine epithelium apoptosis was diagnosed when there was an increase in the individual small, dark, hyperchromatic epithelial cells within the lining of the uterine lumen compared to normal control animals. Uterine hypoplasia was characterized by an overall smaller size, a thinning and less dense stroma of the endometrium, and a reduction in the number of endometrial glands (Figure 41). Squamous metaplasia included areas of flat or stratified squamous non-keratinizing and keratinizing epithelium replacing the uterine columnar lining epithelium and the glandular epithelium (Figure 42). These areas of squamous metaplasia were throughout the length of the uterine horns with the exception of the area near the uterocervical junction. Cystic glandular dilation was diagnosed when the endometrial glands were severely dilated and occurred more frequently throughout the uterine sections compared to control animals (Figure 43). Compression of the lining epithelium was common.

Hyalinization of the stroma was characterized by stroma that had an amphophilic, glassy, and translucent appearance with reduced stromal nuclei (Figure 44).

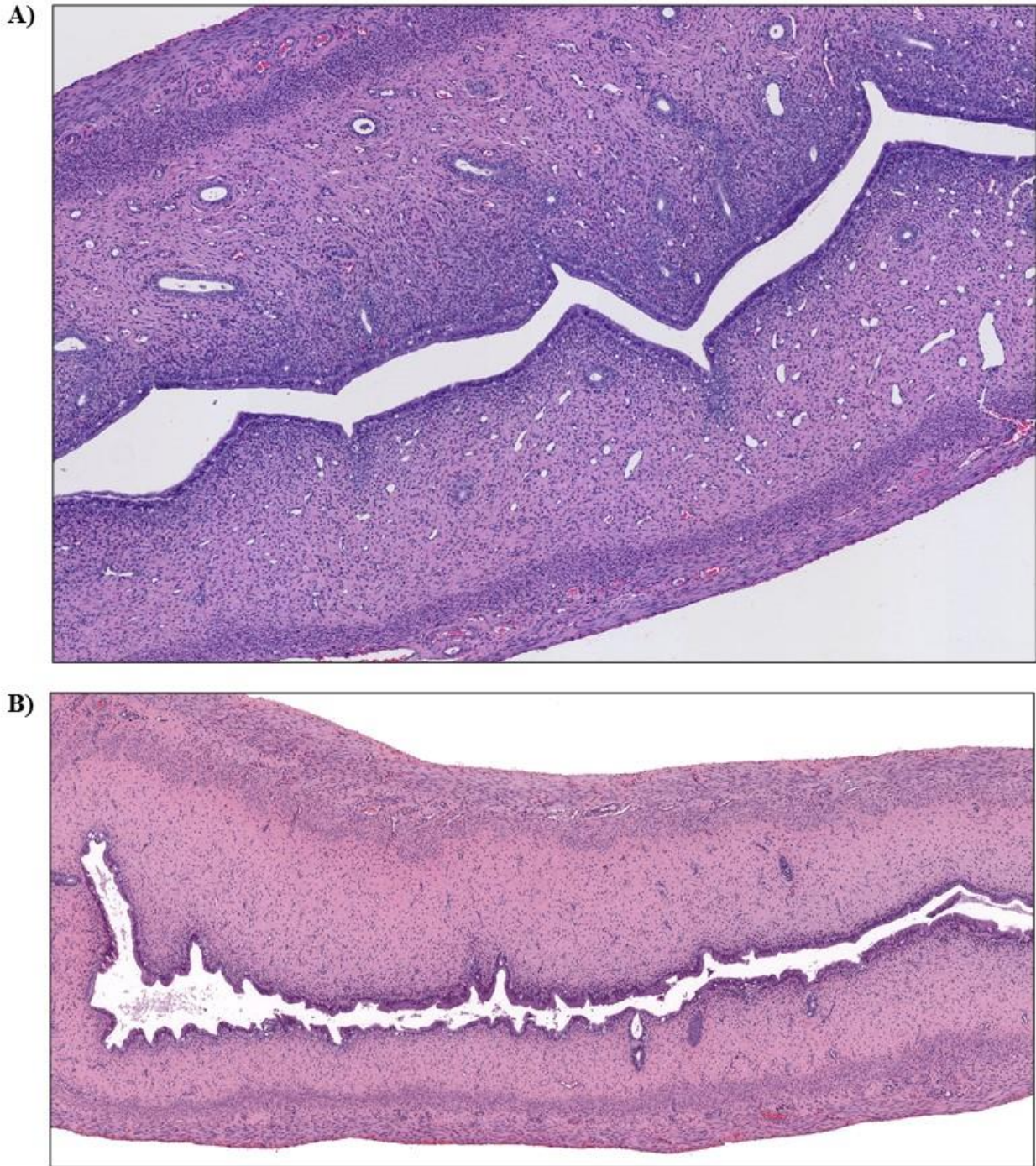


Figure 41. Representative Images of Hypoplasia in the Uterus of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

(A) Normal uterine horn is shown from a control reproductive performance cohort female (4x). (B) Hypoplastic uterine horn is shown from a 3,750 ppm reproductive performance cohort female (4x). Uterine hypoplasia was characterized by an overall smaller uterus size, thinning and less dense endometrial stroma, and a reduction in the number of endometrial glands. H&E = hematoxylin and eosin stain.

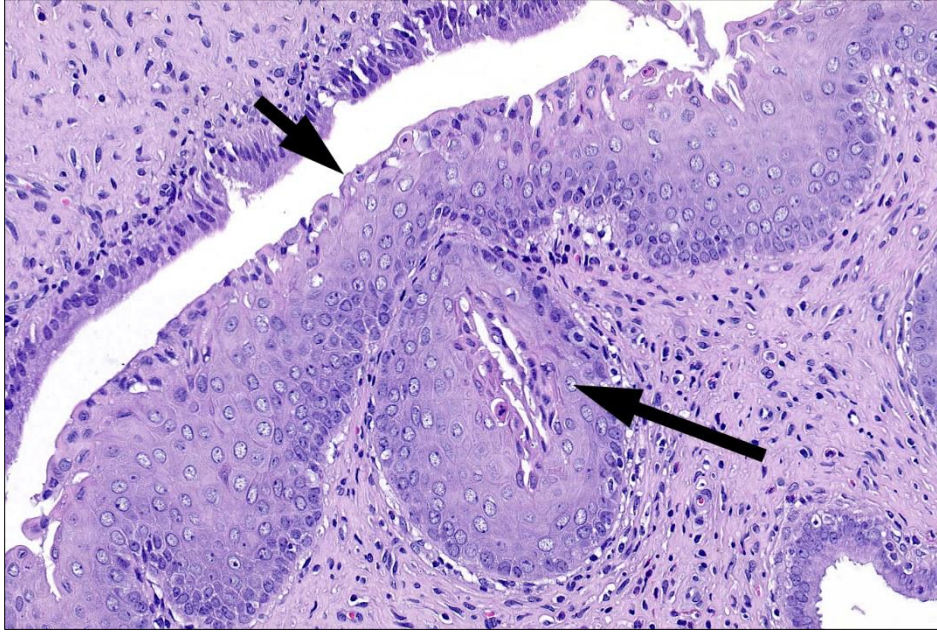


Figure 42. Representative Image of Epithelial Squamous Metaplasia in the Uterus of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

An example of uterine epithelial squamous metaplasia is shown from a 3,750 ppm reproductive performance cohort female (23x). Squamous metaplasia included areas of flat or stratified squamous non-keratinizing and keratinizing epithelium replacing the uterine columnar lining epithelium (short arrow) and the glandular epithelium (long arrow). H&E = hematoxylin and eosin stain.

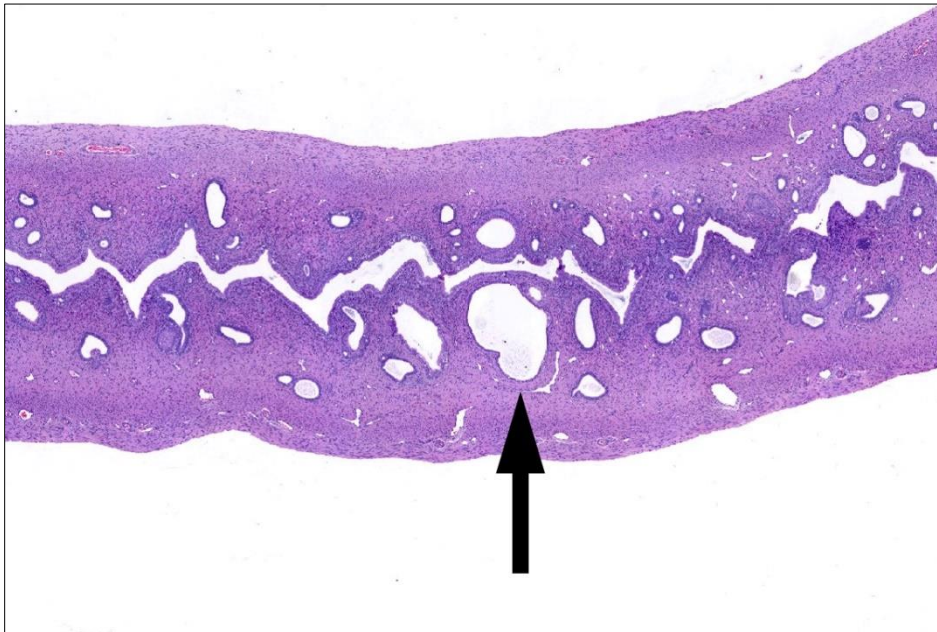


Figure 43. Representative Image of Cystic Glandular Dilation in the Uterus of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

Cystic glandular dilation is present in this uterus from a 3,750 ppm reproductive performance cohort female (2x). This lesion was characterized by endometrial glands that were severely dilated (arrow) compared to control females. H&E = hematoxylin and eosin stain.

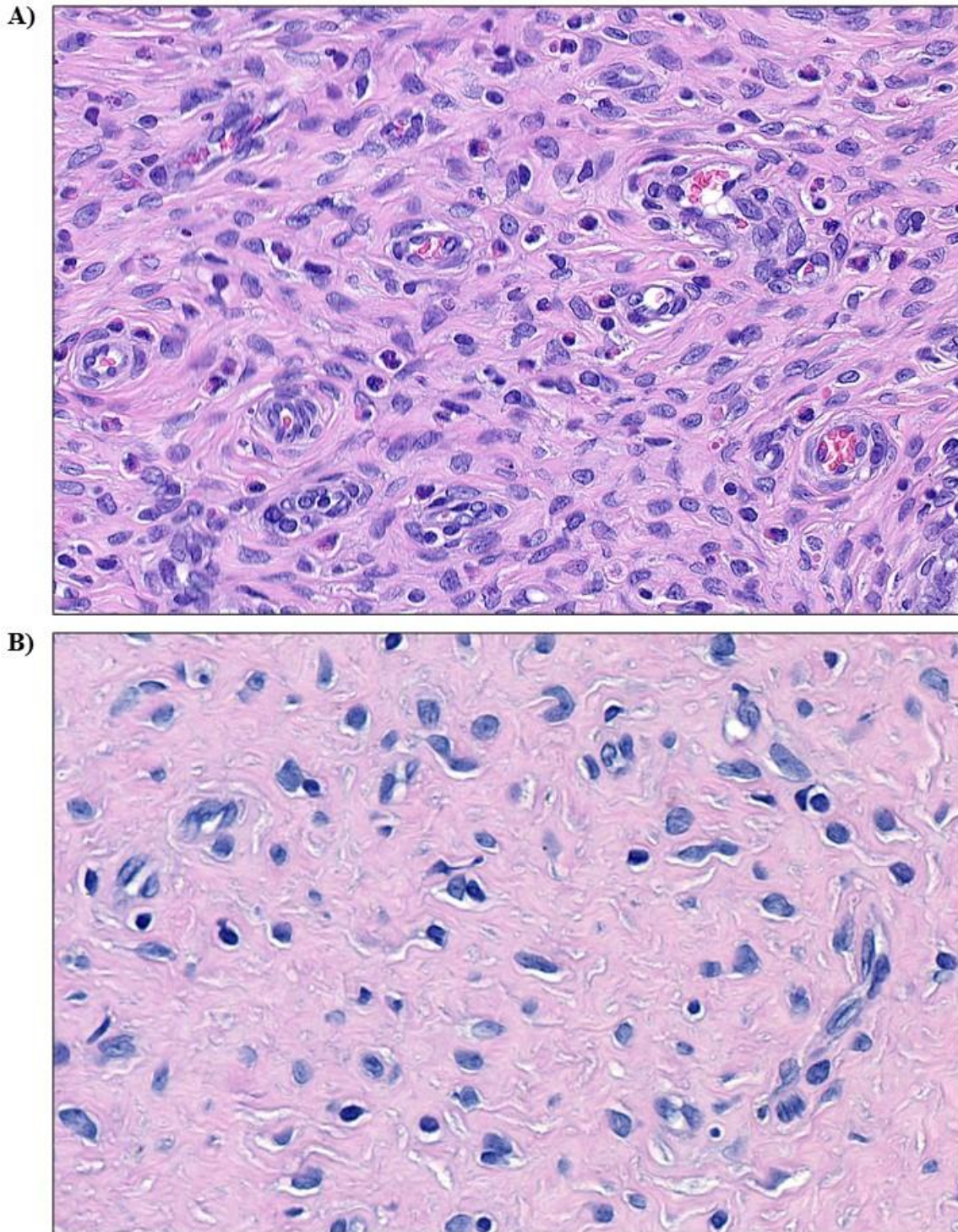


Figure 44. Representative Images of Endometrial Stromal Hyalinization in the Uterus of F₁ Female Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed (H&E)

(A) A high magnification image of normal uterine stroma is shown from a control reproductive performance cohort female (40x). (B) Hyalinization of the endometrial stroma is shown in a 3,750 ppm reproductive performance cohort female (40x). This lesion was characterized by stroma that had an amphophilic, glassy, and translucent appearance with reduced stromal nuclei. H&E = hematoxylin and eosin stain.

F₂ Necropsies

Pups were euthanized on PND 91–93; gross pathology findings and organ weights are reported in Appendix E.

Male Necropsies

For males, there were BPAF-related significant decreases of cauda epididymal weight at 338 (6% below the control group) and 1,125 ppm (19% below the control group) (Table 48). There was no effect of BPAF exposure on percentage of motile sperm, percentage of progressively motile sperm, cauda epididymal sperm concentration (per g cauda epididymis), or on relative testis spermatid head concentration. There were BPAF-related gross findings in the Cowper's glands, LABC, prostate gland, and seminal vesicles (Table 49), and for a few animals in the 1,125 ppm group, these organs were reduced in size.

Table 48. Summary of Reproductive System Parameters of F₂ Male Rats in the Reproductive Performance Cohort Exposed to Bisphenol AF in Feed

Parameter ^a	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. Examined on PND 91–93 ^b	51 (17)	69 (19)	27 (7)	— ^c
Weights (g) ^{d,e}				
Left cauda epididymis	0.211 ± 0.005**	0.198 ± 0.003**	0.171 ± 0.005**	—
Left epididymis	0.584 ± 0.009**	0.551 ± 0.009*	0.501 ± 0.010**	—
Left testis	2.014 ± 0.023**	1.855 ± 0.026**	1.851 ± 0.041**	—
Spermatid Measurements ^f				
Spermatid heads (10 ⁶ /g testis)	130.3 ± 3.0	131.3 ± 2.7	135.7 ± 3.0	—
Spermatid heads (10 ⁶ /testis)	262.3 ± 6.2	243.4 ± 5.6*	250.2 ± 6.0*	—
Epididymal Spermatozoal Measurements ^f				
Sperm motility (%)	64.9 ± 3.7	65.8 ± 1.9	64.5 ± 4.4	—
Sperm progressive motility (%)	45.4 ± 2.6	45.6 ± 1.6	47.9 ± 3.6	—
Sperm (10 ⁶ /g cauda epididymis)	875.3 ± 26.5	880.2 ± 21.2	892.1 ± 34.2	—
Cauda epididymis sperm count (10 ⁶ /cauda epididymis)	186.6 ± 8.6**	175.0 ± 6.2	152.9 ± 8.7*	—

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

PND = postnatal day.

^aData are presented as mean ± standard error.

^bNo. Examined on PND 91–93 = the number of pups examined (number of litters). Spermatid head concentration, epididymis weight, and testis weight for one animal in the control group and one animal in the 338 ppm group were excluded as outliers.

^cNo females were confirmed pregnant for the 3,750 ppm group.

^dStatistical analysis performed using mixed effects models with litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple comparisons.

^eIf there was a lesion in the left organ, the contralateral tissue was taken.

^fStatistical analysis performed using a bootstrapped Jonckheere test for trend, and a Datta-Satten modified Wilcoxon test with Hommel adjustment for pairwise comparisons.

Table 49. Summary of Gross Necropsy Findings in F₂ Male Rats Exposed to Bisphenol AF in Feed^a

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. of Animals Examined ^b	52 (17)	70 (19)	27 (7)	— ^c
Cowper's Gland				
Size, reduced				
Left ^d	1 (1)	0	0	—
Bilateral	1 (1)	0	3 (3)	—
Total	2 (2)	0	3 (3)	—
Levator Ani/bulbocavernosus Muscle Complex				
Size, reduced	0	0	2 (2)	—
Dorsolateral Prostate Gland				
Size, reduced	1 (1)	0	4 (3)	—
Ventral Prostate Gland				
Size, reduced	1 (1)*	0	5 (3)	—
Seminal Vesicles				
Size, reduced				
Bilateral	0*	0	5 (3)	—

Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$.

^aStatistical analysis performed using the Cochran-Armitage test with a Rao-Scott modification for the random effect due to litter.

^bNumber of animals (number of litters) examined for gross lesions.

^cNo females were confirmed pregnant for the 3,750 ppm group.

^dNumber of animals (number of litters) with lesion.

In addition to gross findings, significant decreases of most male reproductive tissue weights, except preputial glands, were observed in both the 338 and 1,125 ppm groups. Terminal mean body weights were not significantly different from the control group for F₂ males (Table 50). Histopathology was not evaluated for the F₂ animals.

Absolute dorsolateral and ventral prostate weights were significantly decreased relative to the control group by 12% and 20% for the 338 and 1,125 ppm groups (dorsolateral), respectively, and by 24% for the 1,125 ppm group (ventral) (Table 50). Relative weights, as compared to body weight, of the dorsolateral prostate were significantly decreased in the 338 and 1,125 ppm groups, and the relative weight of the ventral prostate was significantly decreased in the 1,125 ppm group compared to the control group. The magnitude of the reductions in dorsolateral and ventral prostate weights was larger than the magnitude of the reduction in body weight, suggesting a direct BPAF-mediated suppression of maturation of these tissues. There was no effect of BPAF exposure on ventral prostate weight in the 338 ppm group.

The magnitude of the reduction in weight of the seminal vesicles, Cowper's glands, and LABC was slightly more than the magnitude of the reduction in body weight, suggesting a direct BPAF-mediated suppression of maturation of these tissues. Absolute weights of the seminal vesicles with coagulating glands were significantly decreased by 6% and 16% for the 338 and 1,125 ppm groups, respectively, and relative weights were significantly decreased for the 1,125 ppm group (Table 50).

Absolute weights of the Cowper's glands were significantly decreased by 10% and 22% for the 338 and 1,125 ppm groups, respectively, and relative weights were also significantly decreased for the 338 and 1,125 ppm groups (Table 50).

Absolute weight of the LABC was significantly decreased by 7% and 14% below the control group for the 338 and 1,125 ppm groups, respectively (Table 50). Relative weight of the LABC was significantly decreased for the 1,125 ppm group.

Absolute testis weights were lower by 6%–7% compared with the control group in the 338 and 1,125 ppm groups, with the left testis weight significantly decreased (Table 50). Relative left testis weights were lower than those of the control group for the 338 ppm group, and the 1,125 ppm group was similar to the control group. The absolute epididymal weights for the 338 ppm group were significantly decreased by 6% (left only) and by 12%–14% for the 1,125 ppm group, compared to the control group. Relative left epididymal weights were significantly decreased compared to the control group for the 1,125 ppm group, and there was a negative trend in relative right epididymal weights with exposure concentration. The minimal decreases in testis and epididymal weights (and lack of changes in testis spermatid head concentration and cauda epididymal sperm concentrations) suggest that there was not a strong effect of BPAF on either tissue.

Table 50. Summary of Organ Weights from F₂ Male Rats Following Perinatal Exposure to Bisphenol AF^{a,b}

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Necropsy Body Wt. (g)	388.8 ± 6.6 52 (17) ^c	374.6 ± 5.7 70 (19)	362.8 ± 11.1 27 (7)	— ^d
Dorsolateral Prostate Gland				
Absolute (g)	0.410 ± 0.012** 52 (17)	0.362 ± 0.011** 70 (19)	0.327 ± 0.017** 27 (7)	—
Relative (mg/g) ^e	1.06 ± 0.03** 52 (17)	0.97 ± 0.03* 70 (19)	0.90 ± 0.04** 27 (7)	—
Ventral Prostate Gland				
Absolute (g)	0.528 ± 0.018** 52 (17)	0.514 ± 0.016 70 (19)	0.401 ± 0.022** 27 (7)	—
Relative (mg/g)	1.36 ± 0.05** 52 (17)	1.38 ± 0.04 70 (19)	1.10 ± 0.04** 27 (7)	—
Seminal Vesicles with Coagulating Gland				
Absolute (g)	1.215 ± 0.035** 50 (16)	1.144 ± 0.024* 70 (19)	1.020 ± 0.030** 27 (7)	—
Relative (mg/g)	3.16 ± 0.10** 50 (16)	3.07 ± 0.07 70 (19)	2.82 ± 0.09** 27 (7)	—
Cowper's Glands				
Absolute (g)	0.0898 ± 0.0023** 51 (17)	0.0808 ± 0.0027** 68 (19)	0.0698 ± 0.0033** 27 (7)	—
Relative (mg/g)	0.23 ± 0.01** 51 (17)	0.22 ± 0.01* 68 (19)	0.19 ± 0.01** 27 (7)	—

Bisphenol AF, NTP DART 08

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
Levator Ani/bulbocavernosus Muscle Complex				
Absolute (g)	1.037 ± 0.022** 52 (17)	0.967 ± 0.020** 70 (19)	0.892 ± 0.037** 27 (7)	–
Relative (mg/g)	2.68 ± 0.07* 52 (17)	2.60 ± 0.06 70 (19)	2.46 ± 0.05* 27 (7)	–
Right Testis				
Absolute (g)	1.949 ± 0.045 52 (17)	1.837 ± 0.025 69 (19)	1.829 ± 0.047 27 (7)	–
Relative (mg/g)	5.03 ± 0.10 52 (17)	4.93 ± 0.09 69 (19)	5.07 ± 0.16 27 (7)	–
Left Testis				
Absolute (g)	1.990 ± 0.033* 52 (17)	1.842 ± 0.027** 70 (19)	1.851 ± 0.041* 27 (7)	–
Relative (mg/g)	5.14 ± 0.10 52 (17)	4.95 ± 0.09 70 (19)	5.14 ± 0.16 27 (7)	–
Right Epididymis				
Absolute (g)	0.564 ± 0.014** 52 (17)	0.545 ± 0.009 70 (19)	0.499 ± 0.010** 27 (7)	–
Relative (mg/g)	1.46 ± 0.03* 52 (17)	1.46 ± 0.02 70 (19)	1.38 ± 0.03 27 (7)	–
Left Epididymis				
Absolute (g)	0.584 ± 0.009** 51 (17)	0.551 ± 0.009* 69 (19)	0.501 ± 0.010** 27 (7)	–
Relative (mg/g)	1.51 ± 0.02** 51 (17)	1.48 ± 0.02 69 (19)	1.39 ± 0.03** 27 (7)	–

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData are displayed as mean ± standard error of the litter means.

^bStatistical analysis performed using mixed models with a random effect for litter for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

^cNumber of animals (number of litters). Organs removed as outliers include: seminal vesicles with coagulating gland (from two animals in the vehicle control group), Cowper's gland (one from the vehicle control group and two from the 338 ppm group), right testis (one from the 338 ppm group), and left epididymis (one from the vehicle control group and one from the 338 ppm group).

^dNo females were confirmed pregnant for the 3,750 ppm group.

^eRelative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Female Necropsies

In females, there were BPAF-related significant decreases of terminal body weight (9% and 16% for the 338 and 1,125 ppm groups, respectively) and ovarian weights for the 338 and 1,125 ppm groups relative to the control group (Table 51).

Absolute ovarian weights were significantly decreased compared with the control group by 17%–18% and 31%–33% for the 338 and 1,125 ppm groups, respectively (Table 51). Relative ovarian weights were significantly decreased for the 1,125 ppm group. The magnitude of the

reduction in weights of the ovaries in the 1,125 ppm group was more than the magnitude of the reduction in body weight, suggesting a direct BPAF-mediated suppression of maturation of this tissue. However, there were no gross findings for the ovaries, and histopathology was not evaluated for the F₂ females.

Table 51. Summary of Organ Weights from F₂ Female Rats Following Perinatal Exposure to Bisphenol AF^{a,b}

	0 ppm	338 ppm	1,125 ppm	3,750 ppm
No. Examined ^c	78 (18)	77 (19)	20 (7)	— ^d
Necropsy Body Wt. (g)	243.5 ± 4.3**	221.0 ± 3.8**	204.9 ± 6.4**	—
Right Ovary				
Absolute (g)	0.0680 ± 0.0023**	0.0564 ± 0.0019**	0.0458 ± 0.0013**	—
Relative (mg/g)	0.28 ± 0.01*	0.26 ± 0.01	0.23 ± 0.01*	—
Left Ovary				
Absolute (g)	0.0683 ± 0.0024**	0.0561 ± 0.0022**	0.0470 ± 0.0019**	—
Relative (mg/g)	0.28 ± 0.01**	0.25 ± 0.01	0.23 ± 0.01*	—

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

^aData are displayed as mean ± standard error of the litter means.

^bStatistical analysis performed using mixed models with a random effect for litter for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

^cNo. Examined = the number of pups examined (number of litters).

^dNo females were confirmed pregnant for the 3,750 ppm group.

^eRelative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Genetic Toxicology

BPAF was not mutagenic in *Salmonella typhimurium* strains TA98 and TA100 or in *Escherichia coli* strain WP2 *uvrA* (pKM101) in tests conducted with and without induced male Sprague Dawley rat liver S9 mix. In all three strains, the highest dose was limited by cytotoxicity. However, BPAF was markedly more cytotoxic to the two *S. typhimurium* strains than to the *E. coli* strain (Table D-1).

BPAF was also evaluated using the in vivo peripheral blood micronucleus assay to assess its ability to induce chromosomal damage in the form of structural or numerical alterations. No significant increases in the frequencies of micronucleated immature erythrocytes (PCEs) were observed in male or female rats administered BPAF (338–3,750 ppm) for 17 weeks in dosed feed, and no significant changes in % PCE were observed, suggesting that BPAF exposure did not affect erythropoiesis (Table D-2).

Discussion

The objective of the present study was to characterize the potential for bisphenol AF (BPAF), a fluorinated analog of bisphenol A (BPA) used in the production of polycarbonates, fluoroelastomers, and epoxy resins, to adversely affect any phase of rat development, maturation, or ability to successfully reproduce and/or to cause subchronic toxicity in the F₁ generation.

Along with bisphenol S and bisphenol F, BPAF is considered a “next generation” bisphenol, although it is not currently approved in the United States as a replacement for BPA. A primary concern for this class of chemicals is the potential to act as an endocrine-active substance. Studies in zebrafish and rats indicate that BPA and BPA analogs may have similar toxicity profiles, effects, and estrogenic activity, underscoring potential concerns about health risks that warrant investigation and better toxicological characterization.^{32; 90-95}

Mechanistic studies have shown that BPAF is an estrogen receptor alpha (ER α) agonist^{24; 96; 97} that can activate ER gene transcription⁹⁸ as well as increase uterine size in adult ovariectomized Harlan Sprague Dawley rats when exposed via the oral route.⁹⁹ Compared with endogenous estrogens, BPAF is a weak ER α agonist, although more potent than BPA.⁹⁹ Additional studies suggest BPAF may also act as an estrogen receptor beta (ER β) antagonist, specifically in HeLa cells,^{24; 97; 100} but not HepG2 cells,⁹⁷ and as an androgen receptor antagonist.^{96; 101; 102} This information suggests that BPAF likely activates the ER and antagonizes the androgen receptor to varying degrees.

In this study, Sprague Dawley (Hsd:Sprague Dawley[®] SD[®]) rats were exposed to BPAF in feed using the NTP modified one-generation (MOG) study design. To minimize the potential endocrine activity of phytoestrogens present in rodent diets, a low-phytoestrogen diet, 5K96, was used. Exposure concentration selection was informed by a dose range-finding study in which BPAF-related significant decreases in pup body weights during lactation were noted at concentrations $\geq 7,500$ ppm. Therefore, 3,750 ppm BPAF was chosen as the high-exposure concentration in the MOG study, and exposure concentrations of 338 and 1,125 ppm were selected to aid in identifying potential exposure-response relationships and to avoid excessive overlap of the ingested doses due to increased feed consumption during pregnancy.

Exposure of F₀ females to BPAF via the diet began on gestation day (GD) 6 (implantation). F₁ offspring were exposed to BPAF at the same exposure concentration as their respective dams. Upon weaning, F₁ offspring at each exposure concentration were randomly assigned to one of four cohorts: (1) a reproductive performance cohort (1/sex/litter), (2) a prenatal cohort (1/sex/litter), (3) a subchronic cohort (1/sex/litter from 10 litters), and (4) a biological sampling cohort. Upon sexual maturity, nonsibling F₁ rats allocated to the prenatal and reproductive performance cohorts were paired for mating to evaluate reproductive performance and F₂ prenatal and postnatal development.

In this study, BPAF exposure was associated with lower F₀, F₁, and F₂ mean body weights. The lower F₀ female mean body weights and body weight gains during gestation was associated with a significant exposure concentration-dependent decrease in postnatal day (PND) 1 F₁ pup weights in the 1,125 and 3,750 ppm groups that continued through PND 98. Consequently, the F₁ female mean body weights of both the reproductive performance and prenatal cohorts were lower for the BPAF-exposed groups at the time of cohabitation and through gestation (no

pregnancies were observed for the 3,750 ppm group). A reduction in litter size and fetal or pup weights contributed to the overall lower mean body weights that occurred during gestation for the 1,125 ppm group. For the F₂ pups in the reproductive performance cohort, there was a BPAF-related significant decrease in mean body weights of both 1,125 ppm male and female pups through weaning, but only female postweaning mean body weights were significantly decreased through PND 91 for both the 338 and 1,125 ppm groups.

Interestingly, the lower body weight gains in the F₁ and F₂ generations were not directly correlated with lower absolute feed consumption values (g/animal/day) as these values were variable; relative feed consumption (g/kg/day) was either similar to or significantly increased compared to that of control animals. Although the increases in feed consumption likely represent some feed wastage (palatability issues were noted in both the dose range-finding study and this study), the changes in absolute feed consumption are likely related to the size of the animals as both F₁ and F₂ pup mean body weights were lower at 1,125 (F₁ and F₂) and 3,750 (F₁ only) ppm. It is also possible that the BPAF-related effects on mean body weight and feed consumption could be mediated through ER signaling. Several *in vitro* studies have demonstrated, through induction of luciferase expression, that BPAF has high estrogenic activity.^{24; 96-98} Natural and synthetic estrogens are known to reduce growth and body weight in rodents¹⁰³⁻¹⁰⁵ and have been shown to have effects on the regulation of food intake.^{103; 106} Multigenerational studies with genistein and ethinyl estradiol have also reported decreased body weights.¹⁰⁷⁻¹⁰⁹ Food intake, metabolism, and body fat distribution can all be modulated by estrogens,^{103; 110; 111} and they can act centrally to modulate orexigenic and anorexigenic hormones to increase or decrease appetite, respectively, and energy homeostasis.^{103; 106} This may provide a potential explanation for some of the mean body weight and feed consumption results observed.

BPAF-related changes in reproductive performance were observed at all exposure concentrations. For the 3,750 ppm group, a slight but significant increase in gestation length for F₀ females, a significant decrease in F₁ pup survival (PND 1–4), and a complete absence of pregnant females in the F₁ generation were observed. Similar findings, although to a lesser extent, were observed at lower concentrations in the prenatal cohort and included a significant decrease in the number of F₁ females with live fetuses or live litters, number of corpora lutea, and number of implantation sites in the 1,125 ppm group, which were associated with significant increases in pre- and postimplantation loss values. Significant decreases in the number of corpora lutea and implantation sites were noted for the prenatal cohort females in the 338 ppm group.

Additional BPAF-related changes consistent with both male and female developmental toxicity were observed in the F₁ generation as indicated by changes in organ weights that were associated with gross and microscopic findings. There was a BPAF-related significant increase in relative weights of the lungs, adrenal glands, and thyroid gland and significant decreases in relative weights for the liver and kidney (left) for the 3,750 ppm F₁ males in the subchronic cohort. For the kidney, microscopic findings (mineral lesions along the junction of the cortex and medulla) were observed in the 3,750 ppm group and are a common background finding in female rats,¹¹²⁻¹¹⁴ although they are less common in males and may signify a potential estrogenic effect on the males.¹¹⁵ There were lower male reproductive organ weights with microscopic findings, as well as impacts on andrology parameters, in F₁ males. Most male reproductive tissue weights, except the preputial glands, were lower compared to the control group in F₂ males in both the 338 and 1,125 ppm groups, with lower organ weights at 1,125 ppm correlating with a reduction in size, noted at necropsy. Most organ weight changes noted were more than the magnitude of the

reductions in body weight. This finding, along with the F₁ histopathological observations of hypoplasia, indicates a potential direct BPAF-mediated suppression of maturation of these tissues.

In F₁ males, absolute weights were lower in the dorsolateral prostate, ventral prostate, and seminal vesicles with coagulating glands for the 1,125 and 3,750 ppm groups and in the Cowper's glands and levator ani/bulbocavernosus muscle (LABC) in the 3,750 ppm group. The changes in the 3,750 ppm group correlated with gross observations of reduced size at necropsy and microscopic observations of hypoplasia for the majority of the animals in this group. F₂ males exhibited similar findings in the same tissues as F₁ males in the 338 and 1,125 ppm groups. Organ weight changes that appeared secondary to the effect of BPAF on body weight were noted in F₁ males and included lower absolute weights of the testes, epididymides, and preputial glands in all three exposed groups, but the histopathological findings of germinal epithelium degeneration and Leydig cell atrophy could contribute to the lower testes weights and suggest a more direct effect of BPAF exposure. Relative testis weights were higher in the 3,750 ppm group, but relative epididymal weights were similar to the control animals. In F₂ males, possible secondary effects of BPAF on body weight were observed as lower absolute weights of the testes and epididymides in the 338 and 1,125 ppm groups, as well as lower relative weights of the testis at 338 ppm and epididymides at 1,125 ppm. Histopathology was not performed on the F₂ generation.

BPAF-related changes in andrology parameters for F₁ males were limited to significant decreases in testes and cauda epididymal weights in the 1,125 and 3,750 ppm groups, as well as a significant decrease in cauda epididymal sperm concentration, and a significant increase in testis spermatid head concentration in the 3,750 ppm group. Microscopic findings in the testis at 3,750 ppm included germinal epithelium degeneration, Leydig cell atrophy, and seminiferous tubule spermatid retention, along with duct atrophy and/or duct exfoliated germ cell and duct hypospermia in the epididymis. BPAF-related changes in andrology parameters for F₂ males were limited to significant decreases in cauda epididymal weight at both 338 and 1,125 ppm (histopathology of the organs was not evaluated for the F₂ generation).

In the F₁ females, reproductive toxicity included significant decreases in absolute ovarian weights in the 1,125 and 3,750 ppm groups; relative ovarian weights were also lower compared to the control group. Those observations correlated with gross observations of reduced size and an exposure concentration-related increase in the incidence of hypoplasia characterized by a lack of or reduction in numbers of corpora lutea and reduced numbers of antral and/or growing follicles in the 3,750 ppm group. A significant decrease in the absolute weight of the uterus/cervix/vagina was observed in the 1,125 and 3,750 ppm groups and was correlated with gross observations of reduced size and hypoplasia of the uterus in the 3,750 ppm group. Uterine hypoplasia was characterized microscopically by an overall smaller size, a thinning and less dense stroma of the endometrium, and a reduction in the number of endometrial glands. Other microscopic changes in the uterus included squamous metaplasia, cystic glandular dilation, and hyalinization of the stroma. Changes were also observed in the F₂ females, which displayed significant decreases in absolute ovarian weights (338 and 1,125 ppm), with relative ovarian weights significantly decreased only in the 1,125 ppm group. The magnitude of the reduction in weights of the ovaries in the 1,125 ppm group was more than the magnitude of the reduction in body weight, suggesting a direct BPAF-mediated suppression of maturation of this tissue. However, there were no gross findings for the ovaries, and histopathological analysis was not

conducted for the F₂ females. In the subchronic cohort, significant increases in the relative weights of the thyroid gland and liver were noted in the 3,750 ppm F₁ females. The decreases observed in select organ weights provide supporting evidence of the impacts of BPAF exposure on reproduction and development.

In addition, BPAF-related changes consistent with impaired development included lower mean body weights for all generations, including fetal or pup weights, and reduced litter sizes, as mentioned above, as well as impacts on fetal parameters and developmental markers.

Select developmental landmarks in both males and females were impacted by BPAF exposure. Vaginal opening (VO), testicular descent, balanopreputial separation (BPS), anogenital distance, and areolae and nipple retention were evaluated in both the F₁ and F₂ generations. The time to VO was significantly accelerated in all BPAF-exposed groups for both the F₁ and F₂ generations at all exposure concentrations. In the F₁ generation, the mean day of achieving VO was significantly accelerated by 2, 8, and 8 days in the 338, 1,125, and 3,750 ppm groups, respectively, and by 3 and 10 days in the F₂ females at 338 and 1,125 ppm, respectively. The mean day of testicular descent was not affected in the F₁ generation, although one male in the 1,125 ppm group and 11 males in the 3,750 ppm group did not attain testicular descent by study termination; however, the mean day of testicular descent was significantly delayed by approximately 2 days for the F₂ offspring in the 1,125 ppm group. In addition, 10 F₁ males in the 3,750 ppm group did not attain BPS. The time to BPS was significantly delayed in both the F₁ and F₂ offspring by 4 and 32 days in the F₁ 1,125 and 3,750 ppm groups, respectively, and by 6 days in the F₂ 1,125 ppm group. There was no effect of BPAF exposure on mean anogenital distance on PND 1 for male and female F₁ and F₂ offspring or on retention of areolae or nipples on PND 13 in F₁ and F₂ male offspring.

Additional fetal parameters were impacted by BPAF exposure. Three F₁ females in the 3,750 ppm group had malformations of the vagina; one had no apparent VO, and two had a misshapen vagina. Direct impacts on the reproductive tract were not limited to females as two F₁ males had malformations of the penis in the 3,750 ppm group; one had the os penis visible at the glans, and the second had incomplete BPS. Both males also had reductions in the size of the dorsolateral prostate, ventral prostate, seminal vesicles, testes, and epididymides. Additional impacts on development were limited to an increase in the incidence of dilated and/or misshapen lateral ventricle (brain) in the 1,125 ppm group and increases in the incidences of rudimentary and full lumbar I (L1) ribs in the 338 ppm group and rudimentary L1 ribs in the 1,125 ppm group for the prenatal cohort. These effects on male and female reproductive tract development, pubertal development, and fetal development were considered clear evidence of developmental toxicity.

At study termination, several biochemical and hematological changes were observed in the F₁ generation. Both male and female rats had significant decreases in serum cholesterol concentrations, while serum triglyceride concentrations were significantly increased in female rats and serum bile acid concentrations were significantly decreased in male rats. Bile acid concentrations in female rats, although not significant, were 45% of control animals in the high-dose group. Similar decreases in cholesterol concentrations were also reported in a 28-day BPAF study.^{29; 116} Additionally, triglyceride and total cholesterol content, as well as genes associated with triglyceride and fatty acid synthesis, were decreased in the livers of female mice exposed to BPAF in utero and during lactation.¹¹⁷ These effects are consistent with the reported ER α agonist

activity of BPAF, as estrogen is an important regulator of liver lipid metabolism (including bile acid metabolism) and serum lipoprotein levels. Estrogen suppresses de novo liver lipogenesis, promotes liver secretion of cholesterol into bile, and plays a role in liver cholesterol uptake and reverse cholesterol transport (i.e., cholesterol removal from peripheral tissues and delivery to the feces).¹¹⁸ BPAF has also been shown to decrease liver PPAR- γ expression. PPAR- γ is an important regulator of lipid metabolism and can increase fatty acid storage while inhibiting fatty acid oxidation.¹¹⁷

In addition to the biochemical changes, hematological changes were observed in F₁ female rats and included a significant decrease in erythrocyte count, hemoglobin concentration, and total white blood cell count for the 3,750 ppm group. These hematology changes may have indicated suppression or disruption of hematopoiesis due to chronic stress of exposure¹¹⁹ or may have been a direct effect of BPAF exposure, particularly as it relates to its purported estrogen receptor activity. Estrogen is a known suppressor of hematopoiesis in rodents, particularly erythropoiesis, through mechanisms that are not fully understood involving disruptions in both thymic and non-thymic hematopoietic regulatory pathways.¹²⁰ BPAF has also been shown to cause perturbations in red blood cell membranes and to enhance eryptosis.^{121; 122} Eryptosis is a key process for the removal of damaged or aged erythrocytes from circulation. Xenobiotics that enhance eryptosis can cause an accelerated removal of erythrocytes from circulation that may lead to decreases in red blood cell counts or anemia and other disorders.¹²² Erythrocytes exposed to BPAF were shown to have increased cytosolic calcium ion levels, increased phosphatidylserine translocation to the external plasma membrane layer, and increased calpain and caspase-3 activities, all of which are triggers for eryptosis.¹²² Differences between the observed biochemical and hematological effects in male and female rats may be a result of the known sexual dimorphism in erythropoiesis and lipid and bile acid homeostasis as it relates to estrogen (estradiol) and its relatively high endogenous levels in females and lower circulating levels in males.^{118; 123; 124}

Free (parent only) and total (combined parent and conjugated forms) BPAF concentrations were quantified in maternal plasma and fetuses at GD 18 and maternal and pup plasma at lactation day (LD) 4 and LD 28.⁴⁴ In maternal plasma, free and total concentrations increased with exposure concentration; free BPAF concentrations were $\leq 1.61\%$ those of total BPAF, demonstrating considerable first pass metabolism of BPAF following exposure via feed. In both GD 18 fetuses and PND 4 pups, the free BPAF concentrations were higher (130%–571%) than corresponding dam concentrations, demonstrating considerable gestational and lactational transfer of parent BPAF from the mother to offspring. Total BPAF concentrations in GD 18 fetuses and PND 4 pups were lower (1.71%–7.23%) than corresponding concentrations in dams, demonstrating either preferential transfer of free BPAF and/or inability of fetuses and pups to conjugate BPAF. Free BPAF concentrations were 11.7%–53.4% that of corresponding total BPAF concentrations in both GD 18 fetuses and PND 4 pup plasma, and the percentage was greater than that observed for dams ($\leq 1.61\%$). Free and total concentrations in PND 28 pups were similar to LD 28 maternal concentrations, demonstrating direct exposure of pups via feed and indicating that conjugating enzymes are developed in PND 28 pups.⁴⁴ Because the ontogeny of conjugating enzymes in humans is similar to that of rodents, the data from rodent BPAF studies could be useful in predicting human risk from exposure to BPAF.

The presence of free BPAF in fetuses and pups confirmed both gestational and lactational transfer of parent BPAF and highlighted the indirect impacts of BPAF on the developing system through multiple generations at almost every exposure examined in this study. Under similar exposure conditions, findings appear to be more notable in females than males, with the exception of organ weights (e.g., there were significant decreases in postweaning mean body weights for both the 338 and 1,125 ppm groups through study termination for F₂ females but not F₂ males).

In addition, select developmental markers (VO and BPS) were significantly impacted by exposure to BPAF in both F₁ and F₂ animals, with delays in BPS at 1,125 and 3,750 ppm for the F₁ generation and at 1,125 ppm for the F₂ generation. An acceleration in VO was observed at all exposure concentrations in both the F₁ and F₂ females, including at the lowest concentration tested (338 ppm). The largest impact was the delay in BPS in the 3,750 ppm group for the F₁ generation (which included some animals that never achieved separation). Comparatively, the findings in the F₂ generation were greater at the same exposure concentration of 1,125 ppm (e.g., acceleration of VO was more prominent and delays in BPS were longer). This may be due to lower body weights and suppression of maturation of select systems, multiple impacts on the reproductive system, or a combination of these factors. Overall, the impacts of dietary BPAF exposure were consistent with estrogenic action and included adverse impacts on body weights, organ weights, and both reproductive and developmental parameters.

Although several in vitro studies have shown BPAF capable of inducing DNA damage,^{37; 38; 40} other assays designed to measure the heritable effects of DNA damage induced by BPAF, such as the in vivo micronucleus and the bacterial mutation assays conducted by the National Toxicology Program (NTP), gave negative results. This contrast highlights the possible fates of the DNA damage identified in DNA damage assays: 1) the damage may be incorrectly repaired and transmitted as a mutation with the possibility of clonal expansion, 2) the damage may be rapidly repaired, as is often the case, or 3) the cell may be unable to repair the damage and the cell will die, thereby eliminating the DNA damage.

Conclusions

Under the conditions of this modified one-generation (MOG) study, there was *clear evidence of reproductive toxicity* of bisphenol AF (BPAF) in Hsd:Sprague Dawley[®] SD[®] rats based on the increased disruption of estrous cyclicity, the inability of the F₁ generation to reproduce, decreases in F₁ pup survival, and a slight increase in gestation length for F₀ females at the highest dietary exposure concentration and, at lower concentrations, decreases in the number of implants, corpora lutea, and live fetuses or litters.

Under the conditions of this MOG study, there was *clear evidence of developmental toxicity* of BPAF in Hsd:Sprague Dawley[®] SD[®] rats based on the presence of fetal malformations and abnormal histopathology of both the male and female reproductive tract in the F₁ generation, impacts on developmental markers, including accelerated vaginal opening and delayed balanopreputial separation, and lower F₁ and F₂ mean body and organ weights.

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Appendix A. Chemical Characterization and Dose Formulation Studies

Table of Contents

A.1. Procurement and Characterization A-2
A.2. Preparation and Analysis of Dose Formulations..... A-3

Tables

Table A-1. Chromatography Systems Used in the Modified One-Generation Study of Bisphenol AF..... A-4
Table A-2. Preparation and Storage of Dose Formulations in the Modified One-Generation Study of Bisphenol AF A-4
Table A-3. Results of Analyses of Dose Formulations Administered to Rats in the Dose Range-finding Study of Bisphenol AF A-5
Table A-4. Results of Analyses of Dose Formulations Administered to Rats in the Modified One-Generation Study of Bisphenol AF A-6

Figures

Figure A-1. Reference Infrared Absorption Spectrum of Bisphenol AF..... A-7
Figure A-2. ¹H Nuclear Magnetic Resonance Spectrum of Bisphenol AF (Lot 20100425) A-8
Figure A-3. ¹³C Nuclear Magnetic Resonance Spectrum of Bisphenol AF (Lot 20100425) A-9
Figure A-4. Ultraviolet/Visible Spectrum of Bisphenol AF (Lot 20100425)..... A-10

A.1. Procurement and Characterization

Bisphenol AF (BPAF) was obtained from 3B Pharmachem International Co., Ltd (Wuhan, China) in a single lot (20100425) that was used in the dose range-finding and MOG studies. The bulk chemical of BPAF lot 20100425 was received in two batches, which were screened for identification and purity to ensure acceptable quality. Subsequently, the two batches were combined and homogenized by mixing for 5 minutes. The final batch was transferred to 80-oz amber glass bottles sealed with Teflon-lined lids and stored at ambient conditions. Identity, purity, and stability analyses were conducted on the final batch by the analytical chemistry laboratory at MRIGlobal (Kansas City, MO). Reports on analyses performed in support of the BPAF studies are on file at the National Institute of Environmental Health Sciences.

Lot 20100425 of BPAF used in this study was a white powder. The melting point of lot 20100425 was determined to be 162.9°C–163.9°C. The octanol/water partition coefficient (K_{ow}) was determined to be $42,634 \pm 21,044$, which resulted in an average log P of 4.63.

The lot identity was confirmed using infrared (IR), ^1H and ^{13}C nuclear magnetic resonance (NMR), and ultraviolet/visible (UV/Vis) spectroscopies. The IR spectrum was in good agreement with the anticipated structure and the reference spectrum from the National Institute of Advanced Industrial Science and Technology (AIST) (Tokyo, Japan) Spectral Database for Organic Compounds (SDBS No. 21770) for BPAF (Figure A-1). ^1H and ^{13}C NMR spectra (Figure A-2, Figure A-3) were consistent with the anticipated structure and the reference spectrum from The Aldrich Library of ^{13}C and ^1H NMR Spectra Edition 1. The UV/Vis spectrum (Figure A-4) supported the structure and was consistent with the reference spectrum from Sadtler Research Laboratories (Philadelphia, PA) (22247 UV). In addition, direct infusion mass spectrometry (DIMS) and elemental analysis were performed to aid in identity confirmation. DIMS confirmed a molecular weight of 336 g/mol for lot 20100425. Elemental analysis was performed by ICON plc (formerly ICON Development Solutions, LLC, Whitesboro, NY). The relative amount of carbon (54.03%), hydrogen (2.94%), fluorine (33.79%), and nitrogen (0.35%) were within 2% of anticipated ratios.

The moisture content of lot 20100425 was determined by Karl Fisher titration. The purity of lot 20100425 was determined using differential scanning calorimetry (DSC) and high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. In addition, headspace gas chromatography (GC/headspace) was performed to determine residual solvent content. Karl Fisher titration indicated a water content of $0.026 \pm 0.005\%$. The DSC analysis yielded a purity of 100% with a melting point of 161.80°C. The HPLC/UV analysis demonstrated one major peak accounting for 99.86% and one minor peak accounting for 0.13% of the total integrated area (Table A-1, System A). The GC/headspace analysis indicated residual solvent peak responses for methanol, cis-1,2-dichloroethene, pyridine, and tetralin, but these were not present at levels greater than the corresponding peaks in the Class 2 standard mixtures (Table A-1, System B). The overall purity of lot 20100425 was determined to be >99.5%.

Accelerated stability studies were conducted on samples of BPAF stored protected from light in amber vials at frozen (-20°C), refrigerated (5°C), ambient (25°C), and elevated (60°C) conditions. After 2 weeks, samples were analyzed by HPLC/UV (Table A-1, System A). Stability of BPAF was confirmed for at least 2 weeks when stored in sealed glass vials at temperatures from -20°C to 60°C .

Periodic reanalysis of the bulk chemical performed by the study laboratory at RTI International (Research Triangle Park, NC) using HPLC/UV (Table A-1, System C) before, during, and after the animal studies showed no degradation relative to a frozen reference standard.

A.2. Preparation and Analysis of Dose Formulations

Dose formulations were prepared monthly by mixing BPAF with 5K96 Verified Casein Diet feed (Table A-2). For the dose range-finding study, formulations were prepared at concentrations of 0, 937.5, 1,875, 3,750, 7,500, and 15,000 ppm (two sets, November and December 2012). The 15,000 ppm formulation was not prepared in December 2012 as the group was terminated early and the formulation was not required. For the modified one-generation study, formulations were prepared at concentrations of 0, 338, 1,125, and 3,750 ppm (11 sets, April to December 2013). The formulation set prepared on November 18, 2013, included only the 0 ppm formulation. Formulations were stored at approximately 5°C and were considered stable for up to 42 days.

Prior to study start, the homogeneity and stability of the formulations were determined by the analytical laboratory using HPLC/UV (Table A-1, System A). The method of preparation was validated for concentration ranges of approximately 200–10,000 ppm for BPAF in feed. High-dose method verification confirmed that formulations up to approximately 45,000 ppm can be diluted into the validated curve range. Additionally, the optimal extraction solvent was determined to be acidified acetonitrile (99:1, acetonitrile:acetic acid, v:v). Homogeneity was confirmed in 22 kg preparations of dose formulations at 250, 937.5, and 15,000 ppm. Homogeneity was confirmed in 37, 50, and 100 kg preparations of dose formulations at 338 and 3,750 ppm by the study laboratory using HPLC/UV (Table A-1, System C).

Stability of the 250 and 937.5 ppm formulations was confirmed for up to 42 days under refrigerated or frozen conditions while protected from light. A 7-day simulated dose study of the 250 and 937.5 ppm formulations was conducted to determine stability in animal room conditions. The formulations spiked with rodent urine and feces had a recovery of approximately 77% by day 7, when compared to the day 0 determined concentration. However, when samples from a 7-day simulated dose study of 937.5 ppm formulation spiked with rodent urine and feces were analyzed using an acid-digestion method, the recovery increased to 90.8%. These results indicate extensive reversible binding of BPAF to feed in the presence of rodent urine and feces and absence of chemical instability when mixed with feed. These results indicate that 5K96 Verified Casein Diet feed formulations containing BPAF are stable under dosing conditions for up to 7 days.

Analysis of pre- and postadministration dose formulations were conducted throughout the studies by the study laboratory using HPLC/UV (Table A-1, System C). Postadministration samples were collected from the animal room at the end of the exposure period. For the dose range-finding study, all dose formulations were analyzed pre- and postadministration (Table A-3). All preadministration samples were within 10% of the target concentration. Postadministration samples were between 70.0% and 93.6% of the target concentrations, with the 7,500 ppm formulation from December 10, 2012, being the only one within 10%. For the modified one-generation study, preadministration samples were analyzed four times over the course of the study, and postadministration samples were analyzed from the first and last formulations representing all four dose groups (Table A-4). All preadministration samples were within 10% of the target concentration. Postadministration samples were between 78.4% and 92.9% of the

Bisphenol AF, NTP DART 08

target concentrations, with the 338 ppm formulation from September 30, 2013, being the only one within 10%.

Table A-1. Chromatography Systems Used in the Modified One-Generation Study of Bisphenol AF

Chromatography	Detection System	Column	Mobile Phase
System A			
High-performance liquid chromatography	Ultraviolet at 210 nm	Altima C-18, Alltech, 250 mm × 4.6 mm ID, 5 μm particle size	60:40 acetonitrile:water, 1.0 mL/min flow rate
System B			
Headspace gas chromatography	Flame ionization detection at 250°C	Restek, Rxi-624Sil MS, 30 m × 0.32 mm ID, 1.8 μm film thickness	Helium, 2.5 mL/min flow rate
System C			
High-performance liquid chromatography	Ultraviolet at 274 nm	Altima C-18, Alltech, 250 mm × 4.6 mm ID, 5 μm particle size	60:40 acetonitrile:water, 1.0 mL/min flow rate

ID = internal diameter.

Table A-2. Preparation and Storage of Dose Formulations in the Modified One-Generation Study of Bisphenol AF

Preparation
<p>A premix of bisphenol AF (BPAF) (lot 20100425) and LabDiet 5K96 Verified Casein Diet feed was diluted with additional feed to reach the target concentration. To make the premix, an appropriate amount of LabDiet 5K96 Verified Casein Diet feed was weighed into a plastic bag. BPAF was weighed into a small container, then transferred into a large stainless-steel container followed by an equal amount of feed from the plastic bag. The contents of the container were thoroughly mixed with a spatula. The remaining feed was used to wash residual BPAF from the weighing container and sides of the stainless-steel mixing container. The contents were mixed thoroughly using the spatula between additions until all feed was incorporated into the premix. To prepare the formulations from the premix, LabDiet 5K96 Verified Casein Diet feed was weighed into a plastic bag. Feed was transferred to the bottom of an 8-quart twin shell blender. An appropriate amount of premix was added to the blender and also evenly distributed between ports. The remaining blank feed was used to rinse the premix container into the blender. The blender ports were sealed, and the formulation was blended for ~15 minutes using an intensifier bar for the first ~5 minutes.</p>
<p>Chemical Lot Number</p> <p>20100425</p>
<p>Maximum Storage Time</p> <p>42 days</p>
<p>Storage Conditions</p> <p>Stored in sealed plastic bag-lined containers at 5°C (refrigerated)</p>
<p>Study Laboratory</p> <p>RTI International (Research Triangle Park, NC)</p>

Bisphenol AF, NTP DART 08

Table A-3. Results of Analyses of Dose Formulations Administered to Rats in the Dose Range-finding Study of Bisphenol AF

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)^a	Difference from Target (%)
November 12, 2012	November 15–20, 2012	0	BLOQ	NA
		937.5	980	5.0
		1,875	1,810	-3.5
		3,750	3,590	-4.3
		7,500	7,050	-6.0
		15,000	14,500	-3.3
December 10, 2012	December 12–14, 2012	0	BLOQ	NA
		937.5	860	-8.3
		1,875	1,740	-7.2
		3,750	3,470	-7.5
		7,500	7,080	-5.6
Animal Room Samples				
November 12, 2012	January 8–10, 2013	0	BLOQ	NA
		937.5	799	-14.8
		1,875	1,650	-12.0
		3,750	3,030	-19.2
		7,500	5,830	-22.3
		15,000	10,500	-30.0
December 10, 2012	January 23–25, 2013	0	BLOQ	NA
		937.5	673	-28.2
		1,875	1,430	-23.7
		3,750	3,140	-16.3
		7,500	7,020	-6.4

BLOQ = below the limit of quantification; NA = not applicable.

^aAverage of triplicate analyses.

Bisphenol AF, NTP DART 08

Table A-4. Results of Analyses of Dose Formulations Administered to Rats in the Modified One-Generation Study of Bisphenol AF

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)^a	Difference from Target (%)
April 29, 2013	April 30, 2013	0	BLOQ	NA
		338	305	-9.8
		1,125	1,040	-7.6
		3,750	3,500	-6.7
July 15, 2013	July 18, 2013	0	BLOQ	NA
		338	322	-4.7
		1,125	1,080	-4.1
		3,750	3,580	-4.5
September 30, 2013	October 1–2, 2013	0	BLOQ	NA
		338	315	-6.8
		1,125	1,110	-1.3
		3,750	3,810	2.0
December 16, 2013	December 17–18, 2013	0	BLOQ	NA
		338	324	-4.1
		1,125	1,120	-0.4
Animal Room Samples				
April 29, 2013	June 19–21, 2013	0	BLOQ	NA
		338	296	-12.4
		1,125	882	-21.6
		3,750	2,950	-21.3
September 30, 2013	November 13–19, 2013	0	BLOQ	NA
		338	314	-7.1
		1,125	951	-15.5
		3,750	3,050	-18.7

BLOQ = below the limit of quantification; NA = not applicable.

^aAverage of triplicate analyses.

Bisphenol AF, NTP DART 08

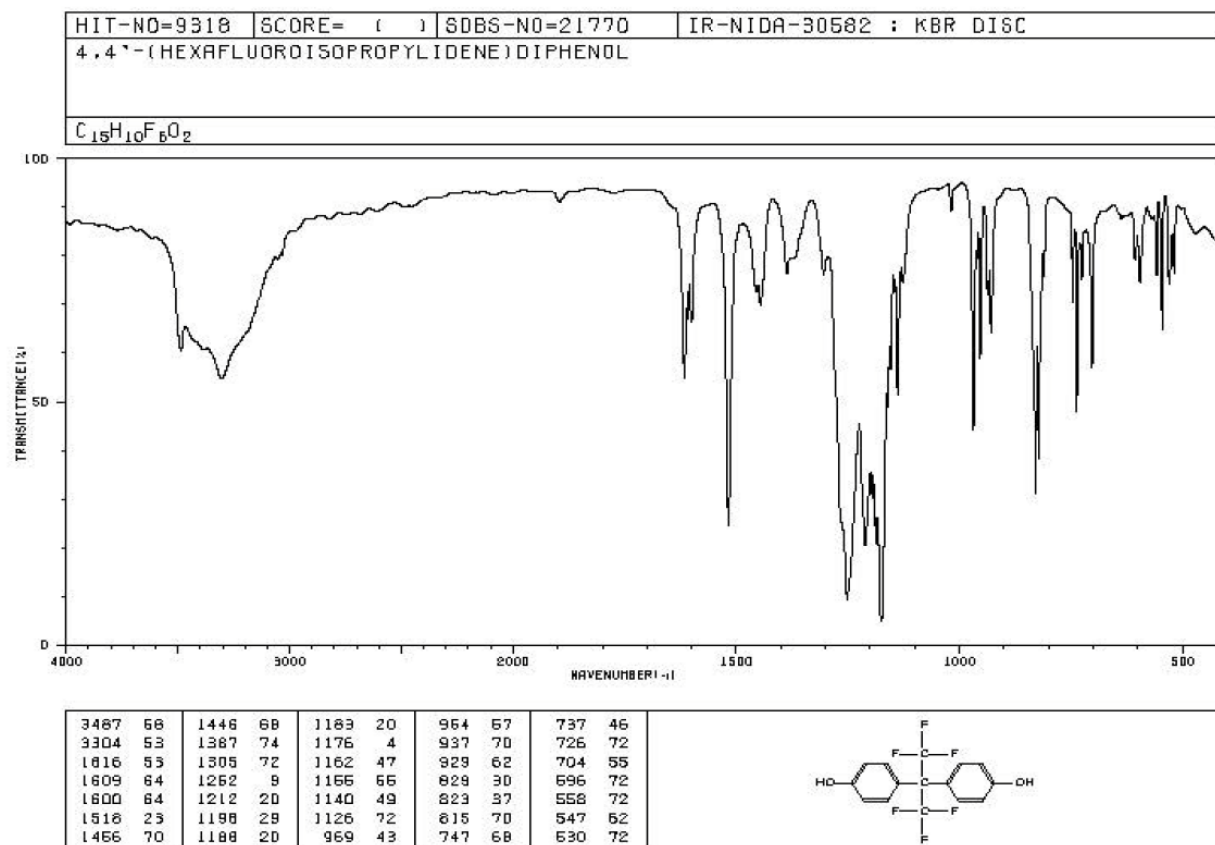


Figure A-1. Reference Infrared Absorption Spectrum of Bisphenol AF

Bisphenol AF, NTP DART 08

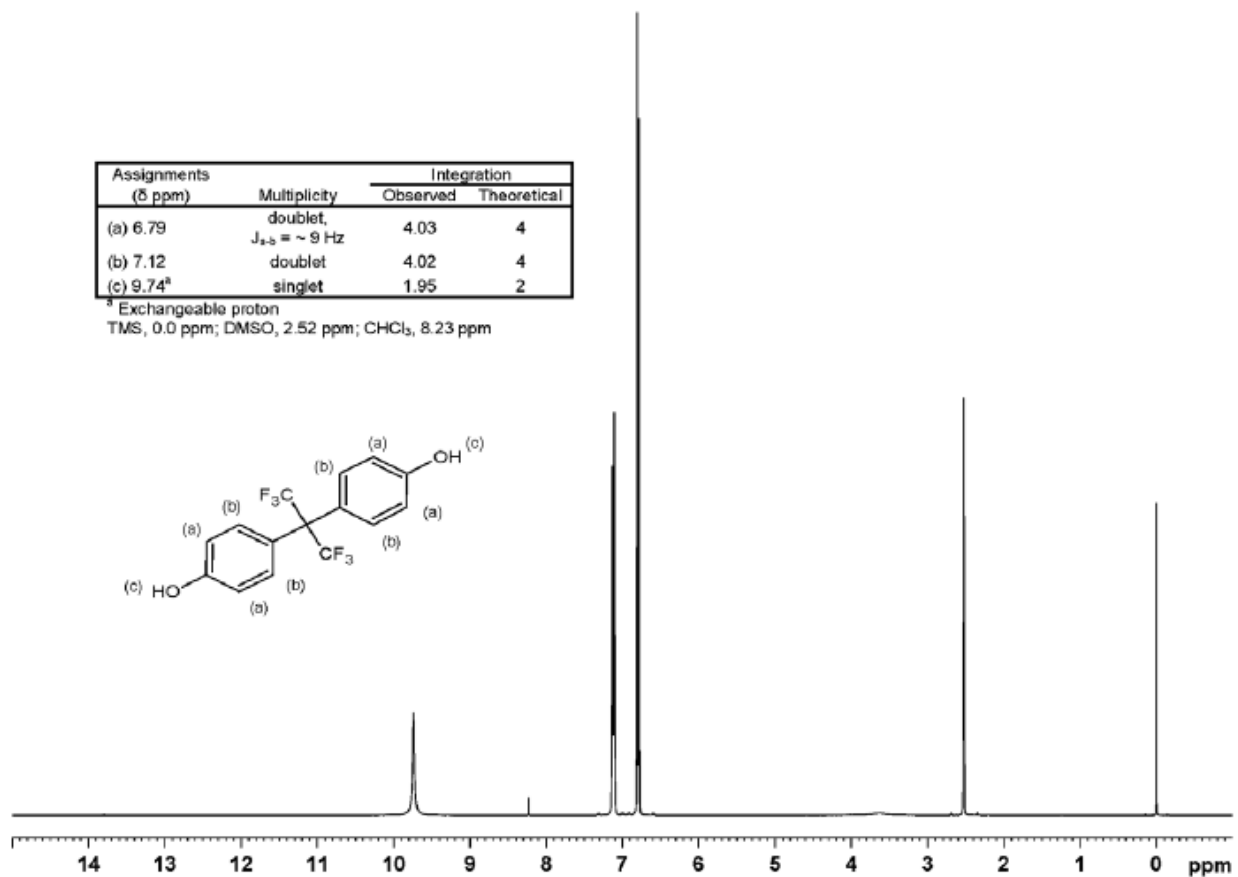


Figure A-2. ¹H Nuclear Magnetic Resonance Spectrum of Bisphenol AF (Lot 20100425)

Bisphenol AF, NTP DART 08

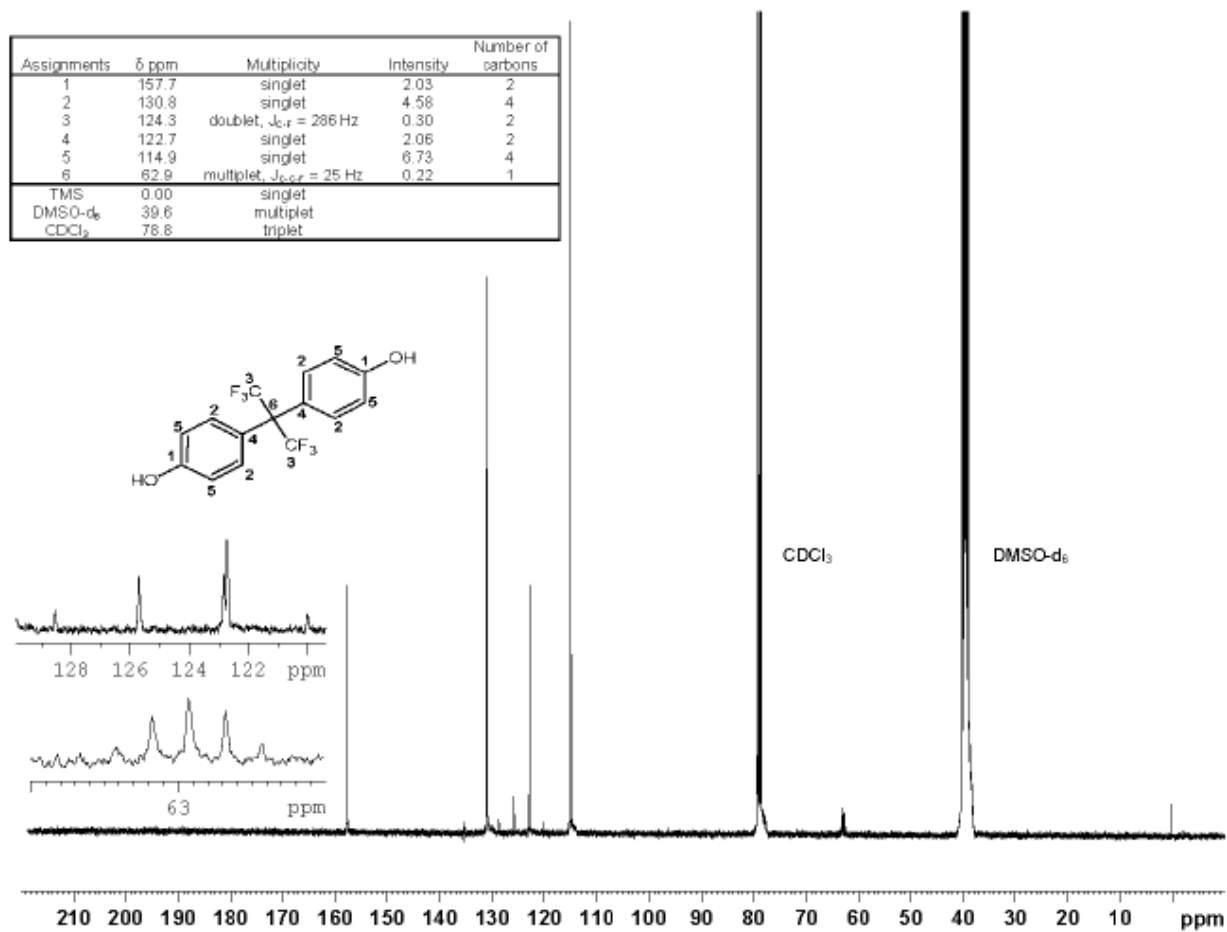


Figure A-3. ^{13}C Nuclear Magnetic Resonance Spectrum of Bisphenol AF (Lot 20100425)

Bisphenol AF, NTP DART 08

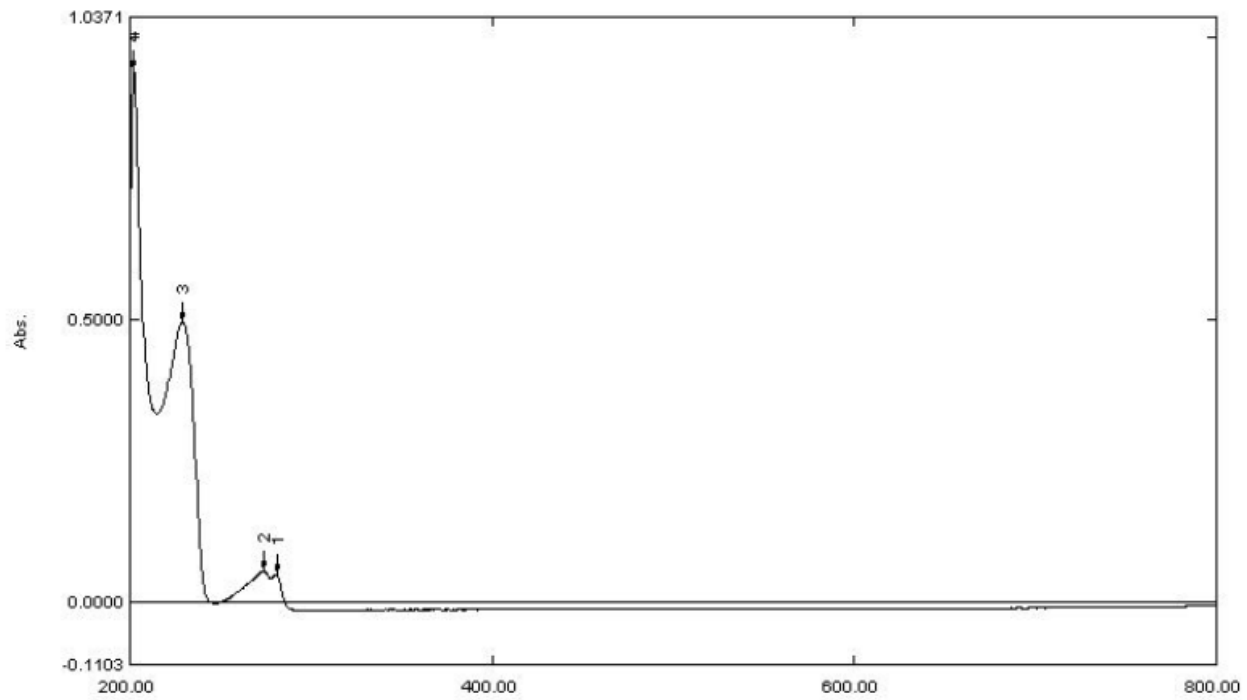


Figure A-4. Ultraviolet/Visible Spectrum of Bisphenol AF (Lot 20100425)

Appendix B. Ingredients, Nutrient Composition, and Contaminant Levels in 5K96 Rat Ration

Tables

Table B-1. Nutrient Composition of 5K96 Rat Ration.....	B-2
Table B-2. Contaminant Levels in 5K96 Rat Ration.....	B-2

Bisphenol AF, NTP DART 08

Additional information on ingredients, vitamins, and minerals in the 5K96 rat diet can be found online.¹²⁵

Table B-1. Nutrient Composition of 5K96 Rat Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	20.74 ± 0.3050	20.4–21.1	5
Crude Fat (% by Weight)	4.34 ± 0.5899	3.5–5.1	5
Crude Fiber (% by Weight)	3.158 ± 0.2216	2.82–3.41	5
Ash (% by Weight)	5.826 ± 0.1627	5.56–5.96	5
Vitamins			
Vitamin A (IU/kg)	14,936 ± 7,602	1,480–19,800	5
Thiamine (ppm)	17.82 ± 1.9071	15.8–20.5	5
Minerals			
Calcium (%)	1.120 ± 0.0982	0.949–1.19	5
Phosphorus (%)	0.891 ± 0.0615	0.795–0.952	5

Table B-2. Contaminant Levels in 5K96 Rat Ration

Contaminant	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.3886 ± 0.0581	0.291–0.438	5
Cadmium (ppm)	0.0403 ± 0.0051	0.0339–0.046	5
Lead (ppm)	0.2866 ± 0.1347	0.15–0.511	5
Mercury (ppm)	0.0127 ± 0.0036	0.01–0.0177	5
Selenium (ppm)	0.4338 ± 0.0503	0.346–0.471	5
Aflatoxins (ppb) ^a	<2.0	–	5
Nitrate Nitrogen (ppm) ^b	17.14 ± 3.0778	13.1–20.6	5
Nitrite Nitrogen (ppm) ^{a,b}	<1.0	–	5
BHA (ppm) ^{a,c}	<1.0	–	5
BHT (ppm) ^{a,c}	<1.0	–	5
Aerobic Plate Count (CFU/g)	<10.0	–	5
Coliform (MPN/g)	<3.0	–	5
<i>Escherichia coli</i> (MPN/g)	<10.0	–	5
<i>Enterobacteriaceae</i> (MPN/g)	<3.0	–	5
Total Nitrosamines (ppb) ^d	4.48 ± 4.1578	0–10.5	5
N-Nitrosodimethylamine (ppb) ^d	2.74 ± 3.3968	0–7.8	5
N-Nitrosopyrrolidine (ppb) ^d	1.74 ± 1.1104	0–2.7	5
Pesticides (ppm)			
α-BHC ^a	<0.01	–	5
β-BHC ^a	<0.02	–	5

Bisphenol AF, NTP DART 08

Contaminant	Mean ± Standard Deviation	Range	Number of Samples
γ-BHC ^a	<0.01	–	5
δ-BHC ^a	<0.01	–	5
Heptachlor ^a	<0.01	–	5
Aldrin ^a	<0.01	–	5
Heptachlor Epoxide ^a	<0.01	–	5
DDE ^a	<0.01	–	5
DDD ^a	<0.01	–	5
DDT ^a	<0.01	–	5
HCB ^a	<0.01	–	5
Mirex ^a	<0.01	–	5
Methoxychlor ^a	<0.05	–	5
Dieldrin ^a	<0.01	–	5
Endrin ^a	<0.01	–	5
Telodrin ^a	<0.01	–	5
Chlordane ^a	<0.05	–	5
Toxaphene ^a	<0.10	–	5
Estimated PCBs ^a	<0.20	–	5
Ronnel ^a	<0.01	–	5
Ethion ^a	<0.02	–	5
Triethion ^a	<0.05	–	5
Diazinon ^a	<0.10	–	5
Methyl Chlorpyrifos	0.0625 ± 0.0295	0.0464–0.115	5
Methyl Parathion ^a	<0.02	–	5
Ethyl Parathion ^a	<0.02	–	5
Malathion ^a	<0.02	–	5
Endosulfan I ^a	<0.01	–	5
Endosulfan II ^a	<0.01	–	5
Endosulfane Sulfate ^a	<0.03	–	5

All samples were irradiated. BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.

^aAll values were below the detection limit. The detection limit is given as the mean.

^bSources of contamination include alfalfa, grains, and fish meal.

^cSources of contamination include soy oil and fish meal.

^dAll values were corrected for percent recovery.

Appendix C. Sentinel Animal Program

Table of Contents

C.1. Methods.....	C-2
C.2. Results.....	C-2

Tables

Table C-1. Methods and Results for Sentinel Animal Testing in Male and Female Rats .C-	
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3

C.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that could affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicological evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or exposed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals are from the same production source and weanling groups as the animals used for the studies of test compounds.

For these dose range-finding and modified one-generation studies, blood samples were collected from each sentinel animal and allowed to clot, and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter* species. All samples were processed appropriately with serology and *Helicobacter* testing performed by IDEXX BioResearch (formerly Rodent Animal Diagnostic Laboratory [RADIL], University of Missouri), Columbia, MO, for determination of the presence of pathogens. Evaluation for endo- and ectoparasites was performed in-house by the testing laboratory.

The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed (Table C-1).

C.2. Results

All test results were negative.

Table C-1. Methods and Results for Sentinel Animal Testing in Male and Female Rats

Collection Time Points	Dose Range-finding Study			Modified One-Generation Study					
	Quarantine	Study Termination	Quarantine	1 Month After Arrival	16 Weeks After Arrival	13 Weeks After Birth ^a	22 Weeks After Birth ^a	32 Weeks After Birth ^a	Study Termination
Number Examined (Males/Females)^b	0/5	0/5	0/5	0/5	0/5	5/0	5/0	5/0	0/5
Method/Test									
Multiplex Fluorescent Immunoassay (MFI)									
Kilham rat virus (KRV)	–	–	–	–	–	–	–	–	–
<i>Mycoplasma pulmonis</i>	–	–	–	–	–	–	–	–	–
Pneumonia virus of mice (PVM)	–	–	–	–	–	–	–	–	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	–	–	–	–	–	–	–	–	–
Rat minute virus (RMV)	–	–	–	–	–	–	–	–	–
Rat parvo virus (RPV)	–	–	–	–	–	–	–	–	–
Rat theilovirus (RTV)	–	–	–	–	–	–	–	–	–
Sendai	–	–	–	–	–	–	–	–	–
Theiler's murine encephalomyelitis virus (TMEV)	–	–	–	–	–	–	–	–	–
Toolan's H-1	–	–	–	–	–	–	–	–	–
Immunofluorescence Assay (IFA)									
<i>Pneumocystis carinii</i>	–	NT	–	NT	NT	NT	NT	NT	NT
Pneumonia virus of mice (PVM)	NT	NT	NT	NT	–	NT	NT	NT	NT
Polymerase Chain Reaction (PCR)									
<i>Helicobacter</i> species	–	–	NT	–	–	–	–	–	–

– = negative; NT = not tested.

^aMale rats born at RTI International.^bAge-matched nonpregnant females.

Appendix D. Genetic Toxicology

Table of Contents

D.1. Data Evaluation Protocol	D-2
D.2. Bacterial Mutagenicity	D-2
D.3. Micronucleus Assay	D-4

Tables

Table D-1. Mutagenicity of Bisphenol AF in Bacterial Tester Strains.....	D-3
Table D-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Modified One-Generation Study of Bisphenol AF.....	D-5

D.1. Data Evaluation Protocol

The National Toxicology Program (NTP) considers biological as well as statistical factors to determine an overall assay result. For an individual assay, the statistical procedures for data analysis are described in the following protocols. There have been instances, however, in which multiple samples of a chemical were tested in the same assay, and different results were obtained among these samples and/or among laboratories. In such cases, all the data are critically evaluated with attention given to possible protocol variations in determining the weight of evidence for an overall conclusion of chemical activity in an assay. For in vitro assays conducted with and without exogenous metabolic activation, results obtained in the absence of activation are analyzed separately from results obtained in the presence of activation. The summary table in the abstract of this report presents NTP's scientific judgment regarding the overall evidence for activity of the chemical in an assay.

D.2. Bacterial Mutagenicity

D.2.1. Bacterial Mutagenicity Test Protocol

Testing procedures were modified from those originally reported by Zeiger et al.¹²⁶. Coded samples of bisphenol AF (BPAF) were incubated with the *Salmonella typhimurium* (TA98, TA100) or *Escherichia coli* WP2 *uvrA* (pKM101) tester strains either in buffer or S9 mix (metabolic activation enzymes and cofactors from phenobarbital/benzoflavone-induced male Sprague Dawley rat liver) for 20 minutes at 37°C. Top agar supplemented with *L*-histidine (or tryptophan for the *E. coli* strain) and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine- or tryptophan-independent mutant colonies arising on these plates were counted after incubation for 2 days at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least six doses of BPAF. The highest dose tested was limited by toxicity in all strains. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose-related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed after chemical treatment. No minimum percentage or fold increase is required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background. Results obtained in trials conducted in the absence of S9 activation are not combined with results obtained in the presence of activation; each testing condition is independently evaluated.

D.2.2. Results

BPAF was not mutagenic in *S. typhimurium* strains TA98 and TA100, or in *E. coli* strain WP2 *uvrA* (pKM101) in tests conducted with and without induced male Sprague Dawley rat liver S9

Bisphenol AF, NTP DART 08

mix. In all three strains, the top dose was limited by cytotoxicity. However, BPAF was markedly more cytotoxic to the two *S. typhimurium* strains, compared with the *E. coli* strain (Table D-1).

Table D-1. Mutagenicity of Bisphenol AF in Bacterial Tester Strains^a

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA98					
	0	46 ± 4.0	56 ± 1.9	50 ± 7.2	55 ± 1.3
	4.0	50 ± 6.9	49 ± 4.5	–	–
	8.0	53 ± 4.6	67 ± 9.2	55 ± 7.5	60 ± 3.2
	20.0	48 ± 4.6	58 ± 1.7	48 ± 0.9	58 ± 4.6
	30.0	37 ± 1.7	50 ± 0.9	–	–
	50.0	14 ± 1.5 ^s	26 ± 2.2	41 ± 3.8	68 ± 1.2
	125.0	Toxic	Toxic	36 ± 2.9	39 ± 0.6
	250.0	–	–	18 ± 0.3 ^s	28 ± 1.2
	500.0	–	–	Toxic	7 ± 1.5 ^s
Trial Summary		Negative	Negative	Negative	Negative
Positive Control ^b		523 ± 31.1	618 ± 31.5	1,339 ± 30.4	1,449 ± 23.6
TA100					
	0	95 ± 6.4	134 ± 14.7	103 ± 4.7	139 ± 7.0
	4.0	94 ± 2.3	128 ± 5.2	–	–
	8.0	108 ± 7.0	159 ± 32.4	99 ± 4.7	134 ± 13.1
	20.0	89 ± 5.7	111 ± 3.1	114 ± 6.5	129 ± 10.3
	30.0	85 ± 6.3	102 ± 9.0	–	–
	50.0	3 ± 1.0 ^s	10 ± 4.0 ^s	103 ± 4.9	124 ± 2.6
	125.0	2 ± 1.0 ^s	Toxic	83 ± 9.3	105 ± 7.3
	250.0	–	–	30 ± 2.1 ^s	52 ± 2.3 ^s
	500.0	–	–	26 ± 4.0 ^s	Toxic
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		709 ± 4.4	761 ± 32.9	711 ± 64.1	610 ± 25.8
<i>Escherichia coli</i> WP2 <i>uvrA</i> (pKM101)					
	0	154 ± 7.4	151 ± 3.8	218 ± 17.2	180 ± 7.8
	125.0	136 ± 4.0	139 ± 8.1	221 ± 11.3	223 ± 13.5
	250.0	109 ± 4.3	115 ± 0.0	186 ± 8.4	178 ± 6.4
	500.0	91 ± 6.9	78 ± 4.8	167 ± 16.4	161 ± 3.3
	750.0	52 ± 10.2 ^s	83 ± 4.7	127 ± 6.3	125 ± 2.3
	1,000.0	52 ± 3.5 ^s	26 ± 1.7 ^s	80 ± 14.2 ^s	108 ± 10.7
	2,000.0	31 ± 6.8 ^s	46 ± 2.2 ^p	32 ± 1.5 ^p	18 ± 1.7 ^x

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		2,425 ± 140.4	3,010 ± 26.5	1,324 ± 24.3	1,585 ± 85.5

^p = precipitate; ^s = slight toxicity; ^x = slight toxicity and precipitate.

^aStudies performed at Integrated Laboratory Systems, LLC. Data are presented as revertants/plate (mean ± standard error) from three plates; 0 µg/plate served as the solvent control (dimethyl sulfoxide).

^bThe positive controls in the absence of metabolic activation were 2-nitrofluorene (TA98), sodium azide (TA100), and 4-nitroquinoline-N-oxide (*E. coli*). The positive controls for metabolic activation were benzo[a]pyrene (TA100) and 2-aminoanthracene (TA98 and *E. coli*).

D.3. Micronucleus Assay

D.3.1. Peripheral Blood Micronucleus Test Protocol

At termination of the studies of BPAF, blood samples (approximately 200 µL) were collected from male and female rats, placed in ethylenediaminetetraacetic acid (EDTA)-coated tubes, and shipped overnight to the testing laboratory. Upon arrival, blood samples were fixed in ultracold methanol using a MicroFlowPLUS Kit (Litron Laboratories, Rochester, NY) according to the manufacturer's instructions. Fixed samples were stored in a -80°C freezer until analysis. Thawed blood samples were analyzed for frequency of micronucleated immature erythrocytes (i.e., reticulocytes or polychromatic erythrocytes [PCEs]) and mature erythrocytes (i.e., normochromatic erythrocytes [NCEs]) using a flow cytometer¹²⁷; both the mature and immature erythrocyte populations can be analyzed separately by employing special cell surface markers to differentiate the two cell types. Because the very young reticulocyte subpopulation (CD71+ cells) can be targeted using this technique, rat blood samples can be analyzed for damage that occurred in the bone marrow within the past 24–48 hours, before the rat spleen appreciably alters the percentage of PCEs in circulation.¹²⁸ In mice, both the mature and immature erythrocyte populations can be evaluated for micronucleus frequency because the mouse spleen does not sequester and eliminate damaged erythrocytes. Damaged erythrocytes achieve steady state in the peripheral blood of mice after four weeks of continuous exposure. Approximately 20,000 PCEs and 1×10^6 NCEs were analyzed per animal for frequency of micronucleated cells, and the percentage of immature erythrocytes (% PCE) was calculated as a measure of bone marrow toxicity resulting from chemical exposure.

Prior experience with the large number of cells scored using flow cytometric scoring techniques^{129; 130} suggests it is reasonable to assume that the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the control group depend on whether the variances among the groups are equal. The Levene test at $\alpha = 0.05$ is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with exposure concentration and the Williams test is used to test for pairwise differences between each exposed group and the control group. In the case of unequal variances, the Jonckheere test is used to test for linear trend and the Dunn test is used for pairwise comparisons of each exposed group with the control group. To correct for multiple pairwise comparisons, the p value for each comparison with the control group is multiplied by the number of comparisons made. In the event that this product is >1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the control group are considered statistically significant at $p \leq 0.025$.

In the micronucleus test, it is preferable to base a positive result on the presence of both a positive trend as well as at least one significantly elevated exposed group compared with the corresponding control group. In addition, historical control data are used to evaluate the biological significance of any observed response. Both statistical significance and biological significance are considered when arriving at a call. The presence of either a positive trend or a single significant exposed group generally results in an equivocal call. The absence of both a trend and any significant differences between exposed groups and the control group results in a negative call. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed (in acute studies), and the magnitudes of those effects.

D.3.2. Results

BPAF was also evaluated in the in vivo peripheral blood micronucleus assay for ability to induce chromosomal damage in the form of structural or numerical alterations; no significant increases in the frequencies of PCEs were observed in male or female rats administered BPAF (338–3,750 ppm) for 17 weeks in dosed feed, and no significant changes in % PCE were observed, suggesting that BPAF exposure did not affect erythropoiesis (Table D-2).

Table D-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Modified One-Generation Study of Bisphenol AF^a

		Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs ^b	P Value ^c	Micronucleated NCEs/1,000 NCEs ^b	P Value ^c	PCEs (%) ^b	P Value ^c
Male								
Exposure Concentration (ppm)								
	0	5	0.62 ± 0.18		0.09 ± 0.03		1.02 ± 0.09	
	338.0	5	0.56 ± 0.10	0.724	0.07 ± 0.02	0.737	1.09 ± 0.07	0.542
	1,125.0	5	0.51 ± 0.15	0.806	0.06 ± 0.01	0.818	1.10 ± 0.08	0.572
	3,750.0	5	0.34 ± 0.07	0.839	0.07 ± 0.01	0.850	1.25 ± 0.11	0.116
	Trend ^d		p = 0.942		p = 0.722		p = 0.083	
Female								
Exposure Concentration (ppm)								
	0	5	0.61 ± 0.09		0.06 ± 0.01		0.87 ± 0.05	
	338.0	5	0.55 ± 0.05	0.803	0.07 ± 0.01	0.596	0.89 ± 0.07	1.000
	1,125.0	5	0.52 ± 0.10	0.874	0.05 ± 0.01	0.684	0.83 ± 0.08	1.000
	3,750.0	5	0.40 ± 0.04	0.899	0.06 ± 0.01	0.719	1.12 ± 0.11	0.067
	Trend		p = 0.976		p = 0.683		p = 0.027	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.

^aStudy was performed at Integrated Laboratory Systems, LLC.

^bData presented as mean ± standard error.

^cPairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).

^dExposure concentration-related trends evaluated by linear regression of the Jonckheere test ($p \leq 0.025$).

Appendix E. Supplemental Data

Tables with supplemental data can be found here: <https://doi.org/10.22427/NTP-DATA-DART-08>.⁸⁹

E.1. Dose Range-finding Study – Rats

E.1.1. Data Tables

I01 – Animal Removal Summary

MOG08002_I01_Animal_Removal_Summary.pdf

I02 – Animal Removals

MOG08002_I02_Animal_Removals.pdf

I03 – Growth Curve

MOG08002_I03_Growth_Curve.pdf

I03C – Growth Curve

MOG08002_I03C_Growth_Curve.pdf

I04 – Mean Body Weights and Survival

MOG08002_I04_Mean_Body_Weights_and_Survival.pdf

I04G – Mean Body Weight Gain

MOG08002_I04G_Mean_Body_Weight_Gain.pdf

I05 – Clinical Observations Summary

MOG08002_I05_Clinical_Observations_Summary.pdf

I05P – Pup Clinical Observations Summary

MOG08002_I05P_Pup_Clinical_Observations_Summary.pdf

I06 – Mean Feed Consumption

MOG08002_I06_Mean_Feed_Consumption.pdf

I08 – Mean Test Compound Consumption

MOG08002_I08_Mean_Test_Compound_Consumption.pdf

PA46 – Summary of Gross Pathology

MOG08002_PA46_Summary_of_Gross_Pathology.pdf

R01 – Multigeneration Cross Reference

MOG08002_R01_Multigeneration_Cross_Reference.pdf

R02 – Reproductive Performance Summary

MOG08002_R02_Reproductive_Performance_Summary.pdf

R03 – Summary of Litter Data

MOG08002_R03_Summary_of_Litter_Data.pdf

R19 – Pup Mean Body Weight Summary

MOG08002_R19_Pup_Mean_Body_Weight_Summary.pdf

R19C – Pup Growth Curve

MOG08002_R19C_Pup_Growth_Curve.pdf

R19G – Pup Mean Body Weight Gain

MOG08002_R19G_Pup_Mean_Body_Weight_Gain.pdf

R20 – Pup Necropsy Summary

MOG08002_R20_Pup_Necropsy_Summary.pdf

E.1.2. Individual Animal Data

Individual Animal Body Weight Data

MOG08002_Individual_Animal_Body_Weight_Data.xlsx

Individual Animal Clinical Observations Data

MOG08002_Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal Consumption Data

MOG08002_Individual_Animal_Consumption_Data.xlsx

Individual Animal Gross Pathology Data

MOG08002_Individual_Animal_Gross_Pathology_Data.xlsx

Individual Animal Litter Data

MOG08002_Individual_Animal_Litter_Data.xlsx

Individual Animal Pup Body Weight Data

MOG08002_Individual_Animal_Pup_Body_Weight_Data.xlsx

Individual Animal Pup Clinical Observations Data

MOG08002_Individual_Animal_Pup_Clinical_Observations_Data.xlsx

Individual Animal Pup Necropsy Data

MOG08002_Individual_Animal_Pup_Necropsy_Data.xlsx

Individual Animal Removal Reasons Data

MOG08002_Individual_Animal_Removal_Reasons_Data.xlsx

Individual Animal Reproductive Performance Data

MOG08002_Individual_Animal_Reproductive_Performance_Data.xlsx

E.2. Modified One-Generation Study – Rats

E.2.1. Data Tables

F₁ All Cohorts Vaginal Cytology Plots

MOG8002B_F1_All_Cohorts_Vagina_Cytology_Plots.pdf

F1 All Cohorts Vaginal Cytology Summary 2020-08-20

MOG08002B_F1_All_Cohorts_Rats_Vaginal_Cytology_Summary_2020_08_20.pdf

F2 Vaginal Cytology Summary 2020-08-20

MOG08002B_F2_Vaginal_Cytology_Summary_2020_08_20.pdf

I01 – Animal Removal Summary

MOG08002B_I01_Animal_Removal_Summary.pdf

I02 – Animal Removals

MOG08002B_I02_Animal_Removals.pdf

I03 – Growth Curve

MOG08002B_I03_Growth_Curve.pdf

I03C – Growth Curve

MOG08002B_I03C_Growth_Curve.pdf

I04 – Mean Body Weight Summary

MOG08002B_I04_Mean_Body_Weight_Summary.pdf

I04G – Mean Body Weight Gain

MOG08002B_I04G_Mean_Body_Weight_Gain.pdf

I05 – Clinical Observations Summary

MOG08002B_I05_Clinical_Observations_Summary.pdf

I05P – Pup Clinical Observations Summary

MOG08002B_I05P_Pup_Clinical_Observations_Summary.pdf

I06 – Mean Feed Consumption

MOG08002B_I06_Mean_Feed_Consumption.pdf

I08 – Mean Test Compound Consumption

MOG08002B_I08_Mean_Test_Compound_Consumption.pdf

PA02R – Neoplastic Lesion Summary with Percent and Litter Incidence

MOG08002B_PA02R_Neoplastic_Lesion_Summary_with_Percent_and_Litter_Incidence.pdf

PA03R – Non-Neoplastic Lesion Summary with Percent and Litter Incidence

MOG08002B_PA03R_Non-Neoplastic_Lesion_Summary_with_Percent_and_Litter_Incidence.pdf

PA05R – Incidence Rates of Neoplastic Lesions with Litter Incidence Systemic Lesions Abridged

MOG08002B_PA05R_Incidence_Rates_of_Neoplastic_Lesions_with_Litter_Incidence_Systemic_Lesions_Abridged.pdf

PA06R – Organ Weights Summary

MOG08002B_PA06R_Organ_Weights_Summary.pdf

PA08R – Statistical Analysis of Neoplastic Lesions with Litter Incidence

MOG08002B_PA08R_Statistical_Analysis_of_Neoplastic_Lesions_with_Litter_Incidence.pdf

PA10R – Statistical Analysis of Non-Neoplastic Lesions and Litter Incidence

MOG08002B_PA10R_Statistical_Analysis_of_Non-Neoplastic_Lesions_with_Litter_Incidence.pdf

PA14 – Individual Animal Pathology Data

MOG08002B_PA14_Individual_Animal_Pathology_Data.pdf

PA18R – Non-Neoplastic Lesion Summary with Mean Severity Grade and Litter Incidence

MOG08002B_PA18R_Non-Neoplastic_Lesion_Summary_with_Mean_Severity_Grade_and_Litter_Incidence.pdf

PA41 – Clinical Chemistry Summary

MOG08002B_PA41_Clinical_Chemistry_Summary.pdf

PA43 – Hematology Summary

MOG08002B_PA43_Hematology_Summary.pdf

PA46R – Summary of Gross Pathology with Litter Incidence

MOG08002B_PA46R_Summary_of_Gross_Pathology_with_Litter_Incidence.pdf

PA48 – Summary of Tissue Concentration

MOG08002B_PA48_Summary_of_Tissue_Concentration.pdf

R01 – Multigeneration Cross Reference

MOG08002B_R01_Multigeneration_Cross_Reference.pdf

R02 – Reproductive Performance Summary

MOG08002B_R02_Reproductive_Performance_Summary.pdf

R03 – Summary of Litter Data

MOG08002B_R03_Summary_of_Litter_Data.pdf

R04 – Anogenital Distance Summary

MOG08002B_R04_Anogenital_Distance_Summary.pdf

R06 – Andrology Summary

MOG08002B_R06_Andrology_Summary.pdf

R09 – Uterine Content Summary

MOG08002B_R09_Uterine_Content_Summary.pdf

R10 – Fetal Defects

MOG08002B_R10_Fetal_Defects.pdf

R11 – Fetal Defect Summary

MOG08002B_R11_Fetal_Defect_Summary.pdf

R13 – Fetal Defect Cross Reference Summary

MOG08002B_R13_Fetal_Defect_Cross_Reference_Summary.pdf

R14 – Developmental Markers Summary

MOG08002B_R14_Developmental_Markers_Summary.pdf

R14C – Time to Attainment Curves for Testicular Descent

MOG08002B_R14C_Time_to_Attainment_Curves_for_Testicular_Descent.pdf

R16 – Pubertal Markers Summary

MOG08002B_R16_Pubertal_Markers_Summary.pdf

R16C – Time to Attainment Curves for Pubertal Markers

MOG08002B_R16C_Time_to_Attainment_Curves_for_Pubertal_Markers.pdf

R19 – Pup Mean Body Weight Summary

MOG08002B_R19_Pup_Mean_Body_Weight_Summary.pdf

R19C – Pup Growth Curve

MOG08002B_R19C_Pup_Growth_Curve.pdf

R19G – Pup Mean Body Weight Gain

MOG08002B_R19G_Pup_Mean_Body_Weight_Gain.pdf

R20 – Pup Necropsy Summary

MOG08002B_R20_Pup_Necropsy_Summary.pdf

Vaginal Cytology Markov Model

MOG08002B_Vaginal_Cytology_Markov_Model.pdf

E.2.2. Individual Animal Data

F₁ Fertility Cohort Vaginal Cytology Plots

MOG8002B_F1_Fertility_Cohort_Vaginal_Cytology_Plots.pdf

F₁ Prechronic Cohort Vaginal Cytology Plots

MOG8002B_F1_Prechronic_Cohort_Vaginal_Cytology_Plots.pdf

F₁ Prenatal Cohort Vaginal Cytology Plots

MOG8002B_F1_Prenatal_Cohort_Vaginal_Cytology_Plots.pdf

Individual Animal Andrology Data

MOG08002B_Individual_Animal_Andrology_Data.xlsx

Individual Animal Body Weight Data

MOG08002B_Individual_Animal_Body_Weight_Data.xlsx

Individual Animal Clinical Chemistry Data

MOG08002B_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Clinical Observations Data

MOG08002B_Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal Consumption Data

MOG08002B_Individual_Animal_Consumption_Data.xlsx

Individual Animal Developmental Markers Data

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Individual Animal Gross Pathology Data

MOG08002B_Individual_Animal_Gross_Pathology_Data.xlsx

Individual Animal Hematology Data

MOG08002B_Individual_Animal_Hematology_Data.xlsx

Individual Animal Histopathology Data

MOG08002B_Individual_Animal_Histopathology_Data.xlsx

Individual Animal Litter Data

MOG08002B_Individual_Animal_Litter_Data.xlsx

Individual Animal Organ Weight Data

MOG08002B_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Pup Body Weight Data

MOG08002B_Individual_Animal_Pup_Body_Weight_Data.xlsx

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Individual Animal Reproductive Performance Data

MOG08002B_Individual_Animal_Reproductive_Performance_Data.xlsx

Individual Animal Teratology Dam Data

MOG08002B_Individual_Animal_Teratology_Dam_Data.xlsx

Individual Animal Teratology Fetal Weight Data

MOG08002B_Individual_Animal_Teratology_Fetal_Weight_Data.xlsx

Individual Animal Teratology Implant Findings Data

MOG08002B_Individual_Animal_Teratology_Implant_Findings_Data.xlsx

Individual Animal Tissue Concentration Data

MOG08002B_Individual_Animal_Tissue_Concentration_Data.xlsx

E.3. Genetic Toxicity Data

BPAF Ames Data

G08002_BPAF_Ames_Data.pdf

BPAF Rat Micronucleus Data

G08002B_BPAF_Rat_Micronucleus_Data.pdf



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